The Jordan Report

Accelerated Development of Vaccines

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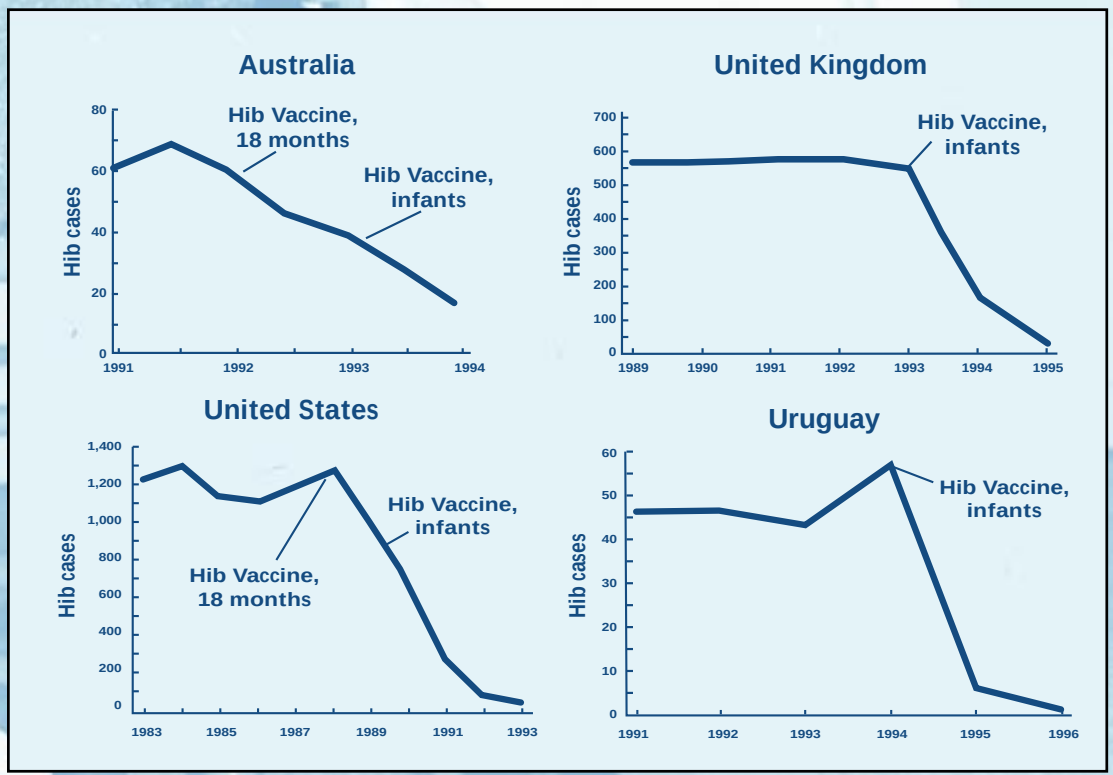

Division of Microbiology and Infectious Diseases
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ABOUT THE COVER

Haemophilus influenzae type b (Hib), a bacterium that routinely lives only in the human oropharynx, is the major cause of bacterial meningitis in children less than 5 years of age, except, as shown on the cover, in countries where Hib conjugate vaccines are routinely used. In the United States, it was estimated that up to 1 in 200 children contracted serious Hib disease by the age of 5. The death rate from this disease ranges from 3 percent in industrialized countries to 30 percent in other areas. Up to 25 percent of survivors of meningitis have long-term neurologic sequelae, including mental retardation and hearing loss. In less industrialized areas, where acute respiratory infections (ARI) are the largest remaining infectious cause of childhood deaths, Hib is the second most common cause of severe ARI.

Prevention strategies have focused on development of effective vaccines, since it was shown in the 1930s that serum bactericidal activity, mediated primarily by antibody to the polysaccharide capsule of the bacterium, was protective. Initial attempts to develop vaccines with the bacterial polysaccharide alone led to products that were immunogenic only in older children, those no longer at risk of disease. However, by chemically conjugating proteins (such as the tetanus and diphtheria toxoids) to the polysaccharide, a series of new-generation vaccines (the Hib conjugate vaccines) were prepared. These vaccines stimulated effective immune reponses in infants when given in routine infant schedules.

Since several randomized trials demonstrated efficacy, the Hib conjugate vaccines have been adopted into routine infant immunization schedules in 25 mostly industrialized countries. The vaccines have been uniformly effective in reducing disease rates by over 95 percent, and in some countries, eliminating disease entirely. Part of the reason for the success of these vaccines has been the reduction in oropharyngeal carriage of the organism that accompanies widespread use of the vaccine. First demonstrated in Finland, where carriage was detected in about 3 percent of children prior to introduction, and 0 percent in children after introduction, reduced carriage in the population reduces the chance of any child (including unvaccinated children) being exposed. This was demonstrated in the United States, where disease rates in children less than 1 year of age declined by nearly 50 percent during a period when only older children were being immunized.

The graphs on the cover show the experience in countries on four continents with these vaccines. The data show numbers of cases of Hib disease or *H. influenzae* meningitis (U.S. data) reported through national surveillance systems (United Kingdom, Uruguay) or large population-based surveillance projects (Sydney area, Australia; 17 States, United States) by year. Cases are all those reported in children less than 5 for U.K., Australia, and the United States, and cases of all ages for Uruguay. The approximate date of national introduction of Hib conjugate vaccines for infant immunization schedules and/or children 18 months and older (as was done initially in Australia and the United States) is shown by the arrows. The surveillance systems from which these data are derived differ in sensitivity and specificity by country, and thus may not represent the entire burden of disease in these countries. Further information on the experience in these countries can be found in the references. The message from these countries is invariably the same—introduction of Hib conjugate vaccines drastically reduced Hib disease.

In spite of the remarkable effectiveness of these vaccines, they are not universally used. In fact, the major burden of Hib disease—ARI and meningitis in the developing world—is largely untouched. A number of factors contribute to the slowness in adopting these vaccines, including lack of appreciation of the burden of disease, issues surrounding introduction into World Health Organization (WHO) Expanded Programme of Immunization programs such as optimal formulation and combinations, and current pricing of the vaccines. The Children's Vaccine Initiative, through WHO, United Nations Children's Fund, and other partners, is currently working on these problems to make these important new vaccines available as rapidly as possible to areas that need them. Specific projects directed at these issues include (1) convening all interested parties for coordination of activities; (2) conducting a series of disease burden studies in areas of the world where the burden of Hib disease is unclear (especially in Asia, eastern Europe, and the Newly Independent States); (3) evaluating countries that have recently introduced Hib vaccines to determine factors associated with the decision to introduce, problems (and solutions) that were encountered, and coordination of data collection on impact on disease; (4) preparing region- and country-specific cost-effectiveness analyses; and (5) making these vaccines affordable to less wealthy countries by evaluating alternative dose schedules, pursuing tiered pricing structures, and seeking other alternative funding methods.

Jay D. Wenger
Children's Vaccine Initiative
World Health Organization

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PREFACE

The quiet success in immunization this year, with the best control over pediatric vaccine-preventable infectious diseases ever reported in the United States, has not gone unnoticed. Age-appropriate coverage of the childhood vaccines is now at a historic high rate, and all the targets set in President Clinton's 1993 Childhood Immunization Initiative have been achieved or exceeded. A renewed emphasis on adult immunization targets the improved use of influenza, pneumococcal, and hepatitis B vaccines along with an enhanced effort to understand the mechanisms that underlie the decline in immune function in the elderly. One of the many goals of this focus is to develop an expanded array of vaccines that will add to our armamentarium to prevent infections and their complications in this growing segment of the population.

Yet despite these advances, our global society and economy highlight the potential effects of emerging and reemerging infectious diseases and the development of antibiotic-resistant organisms. As this Report is going to press, epidemiologists and virologists are in Hong Kong investigating the occurrence of several human cases of influenza due to an avian influenza virus, and efforts are under way to develop a vaccine that could be used to control the spread of this infection. Whether this is a harbinger of epidemics to come or a signal created by improved tools for disease surveillance remains to be seen. But the more important lesson is that we all stand ready to apply rapidly the advances in vaccinology to respond to such threats by developing and testing new vaccines when the need arises.

As the vaccine research and development community has grown, it becomes ever harder to keep up with the expanding scope of the field. *The Jordan Report* began as an annual report to Dr. William Jordan, former Director of the Division of Microbiology and Infectious Diseases, by the Division to track vaccine development as a component of the Program for the Accelerated Development of Vaccines, which was launched in 1981. Since the Division is just one node in a rapidly growing and interdependent network of scientists, industry, government, and health care professionals, the Report can only reflect our view of this dynamic universe. We strive to present as complete a picture as possible and continue to reach out to our colleagues throughout the world of vaccinology to help fill in any gaps that we have missed and those that are bound to develop as the field grows.

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INTRODUCTION

President Clinton's recent proclamation that the development of an AIDS vaccine within the decade is a critical priority for the United States not only started the countdown toward that goal but also served as a reminder of the importance of vaccines in disease prevention and control. Although an AIDS vaccine remains a scientific and technical challenge, the process of developing this vaccine from basic research to full-scale development and evaluation will follow the path of the many vaccines that have been developed and put into widespread use in the United States and around the world. The only prediction one can make about development of an AIDS vaccine is the same one that can be made for the development of any vaccine—the path will not be straight and much will be learned in the various disciplines of "vaccinology" in the process. Despite the rapid incorporation of new technologies in the development of modern vaccines, this remains largely an empiric science. To complement the wide array of vaccine approaches being considered by vaccine companies large and small and to expedite the discovery and development of a safe and effective AIDS vaccine, an AIDS Vaccine Research Center is being established at the National Institutes of Health campus to focus a comprehensive research program and stimulate multidisciplinary research from basic and clinical immunology and virology through to vaccine design and production. The Center will complement the comprehensive extramural research with a center drawn from intramural scientists from the National Institute of Allergy and Infectious Diseases and the National Cancer Institute to others across the NIH.

The global burden of malaria is already severe, with an estimated 300 to 500 million cases of malaria occurring worldwide each year. This past year, an international conference on malaria research in Dakar, Senegal, resulted in the establishment of the Multilateral Initiative on Malaria, a broad-based agenda to improve control of malaria that features vaccine development as a key strategy. At present, no highly efficacious malaria vaccines are available, but there are good reasons to believe that such vaccines are now possible based on evidence of protective immunity in animal models; studies of human volunteers vaccinated with attenuated malaria parasites that protect against challenge with infectious malaria parasites; and, finally, at least one novel candidate vaccine that protected human volunteers who subsequently were experimentally challenged with malaria parasites. The 1997 Kyoto Summit on emissions of greenhouse gases and their potential effect on global warming serves as a reminder of the impact that changing environmental conditions may have on emerging and reemerging infectious diseases. Computer models predict that an increase in global temperature of 3 degrees (C) in the next century could increase the range of malaria-bearing mosquitoes and result in an additional 50 to 80 million cases of malaria each year. In addition, the rapid development and spread of resistant malaria parasites reemphasize the need for a concerted effort in malaria vaccine development.

Mycobacterium tuberculosis, the bacterium that causes tuberculosis, infects one in every three people on Earth, approximately 2 billion people, and last year killed an estimated 2 to 3 million individuals, more than any other single infectious agent. Optimal use of available drugs can make a dent in this burden, but the emergence of a multidrug-resistant form of the bacterium is a growing concern in the United States, as it has now spread to a total of 42 States. This infection is extremely costly to treat, and because treatment is often relatively ineffective, patients remain infectious for much longer than do those receiving proper treatment for drug-susceptible tuberculosis.

The cold-adapted influenza vaccine, originally developed by researchers at the University of Michigan, has been studied by both the U.S. Army and NIH for more than two decades. A Cooperative Research and Development Agreement (CRADA) between NIAID and Aviron began in 1995 for the clinical development of this vaccine, conducted primarily through the NIAID-supported Vaccine and Treatment Evaluation Units (VTEUs). During the past 2 years, the CRADA has moved through 6 clinical protocols involving more than 1,500 patients at the VTEU sites. The multicenter efficacy trial of this vaccine in children at 10 U.S. sites, including the VTEUs, reported excellent protection against circulating influenza strains the first year and is currently evaluating the efficacy of the second-year booster in these children. This rich, intensive collaboration avidly demonstrates the potential yield to the "creative tension" engendered by the intersecting interests of science, government, and industry.

On a contrasting note, the past year has also placed an emphasis on the paradox of vaccine safety. With the highest rates of immunization against childhood diseases ever in the United States, and the lowest rates of the same diseases in the target population, generations of physicians are now being trained who have never seen measles, diphtheria, polio, or tetanus. Even more important, neither have today's parents. In this context, the safety of a vaccine used to prevent disease is held to the highest standards. Yet no vaccine is perfect, forcing difficult scientific, ethical, and public policy decisions. These issues were the focus of the Task Force on Safer Childhood Vaccines, whose report was accepted by the Secretary, Health and Human Services in 1997. Among the recommendations were continued vigilance and Federal coordination through the National Vaccine Program Office.

Coined the "third revolution in vaccine technology," plasmid DNA vaccines continue to be front page news. The papers from

the international meeting held at NIH in 1995 were published this year, and scientific advances continue at a rapid pace. Currently, at least five clinical trials using injections of DNA to stimulate an immune response are ongoing—against HIV, influenza, herpes simplex virus, and T cell lymphoma. As with any new scientific arena, however, progress requires both discovery and the slow, painful, and iterative processes of development. Although early promising results in mice have not been immediately transferable to primates, the potential for effective, safe, and rapidly produced vaccines against viral and parasitic diseases remains high.

The robust scientific base for the accelerated development of vaccines makes possible a number of different target vaccines for a large number of infectious and, potentially, noninfectious diseases. Given this cornucopia of scientific advances, it is indeed timely that the Institute of Medicine of the National Academy of Sciences will produce a report in 1998 on "Vaccine Research and Development Priorities for the 21st Century." Commissioned by the National Institutes of Health, the report will present a framework for evaluating vaccine research and development priorities and identify key vaccines for the next decade.

VACCINE UPDATES

Enteric Infections

Overview

Diarrheal diseases are a major cause of illness in developed countries and are a major cause of both illness and death in developing countries. In the United States, diarrhea is the second most common infectious illness, accounting for one out of every six (16 percent) of all infectious diseases. In some developing countries children have more than 12 episodes of diarrhea per year, and it is a major cause of mortality. Data compiled by the World Health Organization indicate that diarrheal diseases account for 15 to 34 percent of all deaths in certain countries. Conservative estimates place the death toll from diarrheal diseases at 4 million to 6 million deaths per year, with most of these occurring in children of preschool age who are at high risk. In 1991, an epidemic of cholera appeared in the Western Hemisphere for the first time in 100 years; this, as well as the emergence of a new strain of *Vibrio cholerae* in Asia in 1992, underscores the need for both continued surveillance and a research infrastructure capable of rapidly developing vaccines against new or emerging diarrheal infections. The diversity of bacterial and virus infections that cause diarrheal disease complicates accurate surveillance and diagnosis, especially in less developed parts of the world where there is little or no access to modern laboratory procedures. Such surveillance is critical to assess the magnitude of the problem, to establish the need for particular vaccines, and to identify and select sites in affected areas suitable for the testing of new candidate vaccines. As a result, the major focus of NIAID's enteric diseases program will continue to be basic research on the pathogenesis of the organisms responsible for diarrheal diseases. This research will help define the appropriate immune responses to infection and guide efforts to develop new and improved vaccines.

Cholera

More than 100 years after Koch's discovery of the cholera organism, highly effective vaccines remain an elusive goal. The search for a better cholera vaccine is prompted by the results of epidemiological and challenge studies showing that the recovery from natural infection often is followed by solid, long-lasting immunity. The emergence of O139-Bengal in Asia, with its high attack rate in adults, underscores the need for continued surveillance and the development of more effective vaccines against this disease. The goal of this effort is to design safe oral vaccines, composed of either killed or attenuated bacteria, that can provide high levels of protection for several years after the administration of one or more doses of vaccine.

The oral vaccines currently under development are of two types: killed *Vibrio cholerae* bacteria that are combined with purified cholera toxin B subunit (CTB), and strains of *V. cholerae* that are attenuated by virtue of specific gene deletions. Oral vaccination with whole-cell B subunit gave adequate levels of protection (about 50 percent) during at least three cholera seasons in field trials sponsored by the World Health Organization and the United States Agency for International Development in Bangladesh. Multiple doses of the vaccine were required over a 4-month period; unfortunately, young children, the target population for the vaccine, were not well protected. Since that time, the manufacturer (SBL Vaccin AB) has improved the formulation and added a killed *V. cholerae* O139 component to protect against both serotypes. A whole-cell vaccine (mixture of four classical and El Tor strains killed by heat or formalin and not containing CTB) has been produced and tested in Vietnam and showed a protective efficacy of greater than 65 percent against El Tor cholera in both adults and young children. The inactivated vaccines require two doses 1 to 2 weeks apart to achieve this level of protection, but, on the positive side, they do not require a cold chain.

Under NIAID sponsorship, several live-attenuated strains of *V. cholerae* with known genetic deletions have been constructed and tested in volunteers. A vaccine candidate, CVD 103 HgR, lacking the toxic A subunit but retaining the immunogenic B subunit of cholera toxin, has been derived from the classical Inaba strain of *V. cholerae*. This vaccine has been tested in adults and young children in Indonesia and South America and has been shown to be safe and immunogenic. A large-scale efficacy trial, involving more than 60,000 individuals, is under way in Jakarta, Indonesia. Final review of the results of this trial is expected in 1998. CVD 103 HgR has been licensed in some European countries and Canada, and application for U.S. license has been made by the manufacturer, Swiss Serum Vaccine Inc.

Two new live oral vaccines against the El Tor strain of *Vibrio cholerae*, which is the predominant strain in the world at the present time, have been tested in volunteers. Both appear promising as vaccines and are being developed by SSVI and the Virus Research Institute, respectively. The SSVI vaccine has been in clinical trials in the United States, Panama, and Peru in combination with CVD 103 HgR. In these trials it was shown that both strains could be coadministered and an immune response to both biotypes obtained. These groups are also developing live vaccines against the O139 serotype that have been evaluated in U.S. adult volunteers and appear promising. Work also is under way by grantees and by intramural scientists of the National Institute of Child Health and Human Development to produce parenteral cholera vaccines consisting of O antigen conjugated to a variety of

proteins including cholera toxin. Research is also under way to explore the use of *V. cholerae* as an expression vector as the basis for multivalent vaccines.

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Enterohemorrhagic *Escherichia coli* (EHEC)/Shiga Toxin-Producing *E. coli* (STEC)

Shiga toxin-producing *E. coli*, also referred to as enterohemorrhagic *E. coli*, primarily of the O157:H7 serotype, have been in the news a great deal lately. These dangerous strains of *E. coli* express one or both of the Shiga toxins (Stx-I and/or Stx-II) and have been responsible for several recognized food-borne outbreaks in the United States and other developed countries. The Centers for Disease Control and Prevention estimates that as many as 20,000 cases per year occur in the United States. Clinical symptoms can include mild diarrhea, severe abdominal cramping, and bloody diarrhea. Children and the elderly or immunocompromised are at particular risk of developing severe complications including kidney failure due to development of hemolytic uremic syndrome (HUS). Contaminated food products (undercooked ground beef, unpasteurized apple juice, raw milk, sausages, lettuce, and sprouts) have been identified as sources of infection.

Current efforts toward vaccine development are focused on animals (cattle and other ruminants) known to asymptomatically carry these organisms and shed them in their feces. Researchers have shown that immunization of pigs with a genetically modified, nontoxic version (E167Q) of SLT-2e prevents the development of edema disease. Other vaccine approaches target the colonization factor intimin, the protein required for the attaching and effacing lesion characteristic of STEC and EHEC infection in experimental animals. Indeed, if intimin proves to be a good antigen capable of inducing immunity, it would be a useful immunogen against both classes of pathogenic *E. coli*.

Conjugate vaccines targeting the bacterial lipopolysaccharide have also been reported by Dr. Robbins' group at the National Institute of Child Health and Human Development. These vaccines have been discussed as being appropriate for both animal and human use. NIAID grantees have expressed the B-subunit of Stx-I in vaccine strains of *V. cholerae* and shown that experimental rabbits accumulated less fluid in ileal loops challenged with Stx-I than did control animals. Dr. Alison O'Brien is attempting to express intimin in canola, alfalfa, or other animal feed as an edible animal vaccine. Of course, if this strategy were to work in animals, it could also find use as an edible human vaccine.

The sporadic and relatively rare occurrence of infections due to STEC make the usefulness of a vaccine for humans uncertain. A vaccine could be useful during an outbreak to prevent secondary spread to family members or in an institutional or child care environment. If an anti-intimin (or other common antigens) response could be shown to protect against EHEC infection as well as STEC, a stronger case for a vaccine strategy could be made.

Therapeutics for treatment of indivduals infected with STEC are also under development. NIAID grantees and contractors are preparing large quantities of Stx-I and II for toxoiding. If these toxoids prove safe and highly immunogenic in human volunteers, antitoxin antibodies could be purified from donor serum and assessed for their ability to prevent the development of HUS and other serious sequelae in patients presenting with suspected STEC infection. A different antibody-based therapeutic approach is being pursued by Dr. Alison O'Brien in collaboration with scientists at Sunol Corporation in Miami, Florida. They are using recombinant methods to produce "humanized" monoclonal reagents of mouse monoclonals that Dr. O'Brien has shown will neutralize Stx-I and II. These hybrid antibodies, which contain the specific binding variable regions of the original mouse monoclonals with the constant regions of human antibodies, would also be tested for efficacy in preventing the development of the systemic effects of STEC infection.

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Enterotoxigenic *Escherichia coli* (ETEC)

A safe and effective vaccine against ETEC would be useful for travelers and young children in areas of the world where ETEC is endemic. ETEC is second only to rotavirus as the cause of severe dehydrating diarrhea in young children throughout the world. Although surveillance data are difficult to obtain, it is estimated that ETEC causes more than 400 million cases of diarrhea per year and more than 700,000 deaths in children less than 5 years of age. ETEC is also the major cause of traveler's diarrhea, which affects at least 8 million citizens of the United States who travel to endemic regions of the world each year; it causes significant financial hardship in developing countries that rely heavily on tourism.

Volunteer studies have shown that infection with ETEC generates protective immunity against rechallenge with the same strain. On the basis of this observation, several attenuated strains have been developed in an effort to mimic the presentation of important ETEC antigens to the immune system, without inducing disease. Protection correlates with levels of intestinal IgA antibody, specific for the colonization factor antigens (CFA).

The vaccine candidate that is furthest in development has been produced by Swedish investigators. This vaccine is composed of a mixture of five formalin-inactivated ETEC strains, which together express the major CFAs important in human disease, combined with a recombinant cholera toxin B subunit, which will elicit antibody reactive with the ETEC labile toxin (LT). Clinical studies in more than 500 volunteers have demonstrated that the vaccine is safe and immunogenic and capable of generating antibody-secreting cell (ASC) responses equivalent to natural infection in Bangladeshi adults. In studies conducted in Egypt, this vaccine was found to be safe and immunogenic and to induce both ASC and IgG responses in adults. The fact that IgG responses could be measured is important in assessing vaccine response in young children where limited blood samples are available. Subsequent trials have been conducted in progressively younger persons, with comparable safety and immunogenicity results. An ongoing study is being conducted in infants, 6 to 18 months of age.

Other investigators have used attenuated strains of *Shigella* and *Salmonella* to express ETEC colonization factor antigens. Animal experiments with the *Shigella* construct have indicated that an immune response to the expressed CFAs is generated following oral or intranasal administration. These approaches wait, however, for the vector strains themselves to become acceptable vaccine candidates in their own right. At such time vaccine strains may be further engineered as multivalent vaccines by the expression of foreign antigens including ETEC CFAs.

University of Maryland investigators, in cooperation with the Department of Defense, administered colonization factor antigens CS1 and CS3 (CFA/II) that had been encapsulated in biodegradable microspheres to human volunteers. Of 10 volunteers, 5 developed IgA anti-CFA/II ASC following 4 doses of antigen delivered via intestinal tube. Volunteers were challenged with 10^9 colony-forming units (cfu) wild-type ETEC; 10 of 10 unvaccinated controls and 7 of 10 vaccinees developed diarrhea (30 percent vaccine efficacy). Additional human trials are being planned that will use this antigen preparation in combination with a nontoxic mutant *E. coli* labile toxin as an adjuvant.

Drs. Charles Arntzen and John Clements have teamed up on a novel edible vaccine approach. Initial success was obtained with the expression of *E. coli* LT-B in tobacco. Since most people are not fond of eating tobacco, another plant vehicle was needed. Potatoes were chosen, and when expression of LT-B was achieved, animal experiments were performed. Mice fed potatoes containing LT-B developed serum IgG and secretory IgA specific for LT-B. Phase I safety and immunogenicity studies in volunteers have begun at the University of Maryland Vaccine and Treatment Evaluation Unit. Dr. Arntzen's long-term goal is to express antigens in a plant that humans find appetizing, such as banana.

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Helicobacter pylori

It is now well recognized that *Helicobacter pylori* is the main cause of gastric and duodenal ulcers and gastritis, and is a contributing factor for the development of cancers of the stomach. In some developing countries, the infection rate approaches 100 percent of the population, while in the United States as much as 40 percent of the adult population is infected with this organism, although not all infected individuals are symptomatic. It disproportionately affects Hispanic and African Americans. Approximately 10 percent of the United States adult population is affected by peptic ulcer disease (PUD), and an estimated 25 million Americans have had PUD in their lifetimes. At least 90 percent of PUD cases are caused by *H. pylori* infection, but about 70 percent of the U.S. population is unaware of this association. The NIH Consensus Conference held in 1994 recommended that persons diagnosed with ulcer disease be evaluated for their *H. pylori* status and, if found to be infected, that they be treated with a recommended antibiotic therapy designed to eradicate the organism. In 1996, the Food and Drug Administration approved diagnostic products and recommended treatment protocols specifically for *H. pylori* disease. In October 1997, the Centers for Disease Control and Prevention launched a national media campaign designed to educate the public and health care providers about the association between *H. pylori* and ulcer disease and to stress the fact that this is an infectious disease that can be cured by antibiotic therapy.

The usefulness of a vaccine strategy to prevent infection with *H. pylori* is worthy of evaluation. The organism has been shown to be extremely heterogeneous at a genetic level that may make the development of a preventive vaccine difficult. On the other hand, in animal experiments, it has been shown that a vaccine composed of purified urease, a known virulence factor of the organism, can be therapeutic. For this vaccine to be effective, however, the coadministration of the potent mucosal adjuvant, cholera toxin, has been required. Clearly this approach cannot be applied to human vaccination due to the severe diarrheal effects of such toxins. Research is under way, however, on the use of nontoxic mutants of cholera toxin or *E. coli* labile toxin as an adjuvant. In addition to urease, other antigens, combinations of antigens, and killed whole cells or cell extracts are being evaluated by a number of investigators and companies. Other approaches include the expression of *H. pylori* antigens in live-attenuated orally delivered vectors.

The *H. pylori* DNA sequence has been determined by more than one group, but the Institute for Genomic Research was the first to release the sequence to the scientific community. The availability

of these sequence data will permit a detailed analysis of the genome and, predictably, will identify new genes that, by virtue of their similarity to other known bacterial virulence determinants, will become targets for rational vaccine design.

Companies known to be involved in the development of a vaccine against *H. pylori* include OraVax and Astra Research Center, Boston, Massachusetts; Antex Biologics, Rockville, Maryland; IRIS Chiron Biocene, Italy; and Commonwealth Serum Labs, Australia.

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Polio

The number of countries that are free of polio continues to increase each year, and globally, health officials remain optimistic of eradicating the disease by the year 2000. In 1996, 3,997 polio cases were reported worldwide, with a total of 155 countries reporting no cases of polio. Eighteen of the remaining countries reported only 1 to 10 polio cases. That leaves only 27 countries reporting more than 10 cases of polio (with 14 countries not reporting). By World Health Organization (WHO) regions, the numbers of cases reported in 1996 were: African region 1,898, Southeast Asia region 1,116, Western Pacific region 419, Eastern Mediterranean region 373, European region 191, and American region 0. The Pan American Health Organization documented that the last case of paralytic poliomyelitis with a wild-virus isolate in the Western Hemisphere occurred in Peru on August 23, 1991. The successful methods developed during this pioneering regional eradication effort led to a now-standard worldwide eradication strategy of (1) achieving and maintaining high routine vaccine coverage; (2) giving supplemental vaccine doses during "National Immunization Days" (NID) to interrupt wild poliovirus transmission; (3) developing sensitive systems for surveillance; and (4) conducting mopping-up immunization campaigns.

Worldwide immunization is being coordinated by an international coalition of partners including WHO, Rotary International, Centers for Disease Control and Prevention, United Nations Children's Fund, a number of national governments, and many nongovernmental organizations. During 1996 alone, two-thirds of the world's children less than 5 years old received oral polio vaccine. Worldwide coverage with three doses of oral polio vaccine among infants less than 1 year of age has reached 81 percent. In Africa, coverage has increased from 32 percent in 1988 to 60 percent in 1996. During 1996 and 1997, the African region emphasized NIDs, and 74 million children were targeted (three-fourths of all African children less than 5 years old). By mid-1997, roughly 80 percent of this target group was reached in most African countries. Surveillance has also expanded, and surveillance for acute flaccid paralysis is now conducted in 86 percent of the endemic countries. Laboratory confirmation of cases is available through a "Global Laboratory Network for Poliomyelitis Eradication," which now includes 67 national labs, 14 regional labs, and 6 specialized labs. However, the need for repeated contacts with infants to administer the three doses required to immunize fully, as well as the heat sensitivity of the vaccine in tropical settings, continue to remain challenges to the global eradication effort.

The issues related to controlling polio in many developed countries are different from the problems faced by developing countries. Although polio is controlled in such areas, a small number of cases occur each year, and these appear to be associated with use of the attenuated vaccine. The United States currently follows three different polio immunization schedules of either all oral polio vaccine (OPV), all inactivated polio vaccine (IPV), or two IPV followed by two OPV. The sequential IPV/OPV vaccination schedule is intended to reduce vaccine-associated paralytic polio (VAPP) while maintaining individual and population immunity. A study of all VAPP cases from 1980 to 1994 in the United States showed that of the 125 cases (annual mean 8), 76 percent were in immunologically normal OPV vaccinees or contacts, and 24 percent were in immunocompromised persons. Ninety-seven percent of these cases were associated with the first or second dose—1 case per 750,000 children receiving their first dose. Thus, starting immunization with IPV is hoped to establish solid immunity before the first OPV dose. This change to three separate schedules came only after a series of meetings that reviewed substantially differing opinions on the public health impact of altering the highly successful all-OPV schedule that had been in place for decades.

As the world approaches eradication of polio, there have been preliminary meetings to discuss whether there will be a time when all polio immunization could be stopped. This issue is controversial, with some experts recommending continuing OPV, others recommending continuing indefinitely only with IPV, and still others seeing a possibility of stopping all immunization after a "band" period of only IPV. This issue is unresolved and will remain under increasingly intensive discussion.

Further questions about the post-eradication era center on the ability to perform research on wild poliovirus strains in less than biosafety level 4 containment facilities. After eradication, there is concern that the laboratory or the vaccine manufacturing facility would become a source of reintroduction of wild poliovirus into the community. The seed virus for production of IPV is a high-yielding, wild-type poliovirus, and recently, there was a case of accidental transport of the strain from a production facility into the community via an infected but immunized worker. Eventually, if poliovirus immunization is stopped, all poliovirus strains, including vaccine-derived strains, might have to be contained or destroyed. Other unresolved questions about the post-eradication era include: (1) is reintroduction possible from persistently vaccine-infected, immune-suppressed individuals; (2) could these persistent infections be controlled with immune globulins or antivirals; (3) which vaccine would be used if a reemergence occurred; (4) which vaccine(s) will be needed in the post-eradication age; (5) how will these vaccines be produced if all stocks are destroyed or high-containment production facilities are required; and (6) would polio bio-terrorism become an important concern?

The National Institute of Allergy and Infectious Diseases currently funds several extramural basic research projects on the virological and immunological aspects of polio. One goal of this work is to apply the knowledge obtained to make better vaccines that will be (1) genetically stable and not revert to a more neurovirulent form, and (2) more efficient and efficacious, especially when used in tropical and developing regions of the world.

Several major NIAID-supported discoveries have added greatly to the knowledge of poliovirus, as well as other RNA viruses. Molecular studies have been substantially advanced by the development of quick, reliable nucleic acid sequencing methods and the construction of a cDNA infectious clone of poliovirus. The changes in viral nucleic acid that occur during vaccine reversion to virulence have been defined, and a number of studies are examining the basis of viral virulence and attenuation.

The detailed study of viruses has always been hindered by the fact that viruses must invade a host and replicate within living cells; however, research supported by NIAID shows that it is possible to induce the *de novo* synthesis of infectious poliovirus in a cell-free, test-tube system. This system has provided a number of new research approaches to study virus replication.

Another major breakthrough was the ability to insert into mice the human gene responsible for producing the receptor for human poliovirus. Since such "transgenic" mice are able to make the receptor for polio virus, they become susceptible to infection and develop a paralytic-like disease. These new mice have helped advance research focusing on the pathogenesis of viruses.

These discoveries are of great significance not only for the study of poliovirus, but also for research on other viruses. As a model, polio research has led to major breakthroughs, particularly in other RNA viral systems. Non-polio enteroviruses will remain a problem even after eradication. In a recent study of more than 3,200 cases between 1993 and 1996 in the United States, echoviruses 9, 30, 6, and 11 were commonly isolated, as were coxsackieviruses B5, A9, and B2. This group of viruses requires intensified research. The knowledge derived from poliovirus studies will be of great value in the development of new vaccines or antiviral drugs against many other now difficult-to-study RNA viruses.

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Rotavirus

Rotavirus is the leading cause of severe diarrheal disease of infants in both developed and developing countries. It is particularly satisfying, therefore, to report that licensure of the first vaccine against rotavirus is likely in the United States within the next year. Wyeth-Ayerst has applied to the Food and Drug Administration for licensure of the tetravalent human-rhesus reassortant vaccine, which was developed by NIAID intramural scientists. Results of clinical trials indicate that this vaccine should prevent 50 to 60 percent of all rotavirus-induced diarrhea and 90 percent of serious dehydrating diarrhea due to rotavirus. Another reassortant vaccine developed by NIAID grantees, who combined bovine and human viruses, has been tested by Merck, Sharp, and Dohme and Company. This vaccine shows protection in human trials similar to that of the Wyeth vaccine and is being pursued actively by Merck but has not yet been submitted for licensing. Both the Wyeth and Merck vaccines contain G serotypes 1-4 that predominate in the United States.

Both vaccines have limitations, however, that stimulate additional research. Intramural scientists have developed a cold-adapted human rotavirus candidate vaccine that is currently being evaluated in phase I clinical studies for safety. A virus isolated from a naturally infected, asymptomatic child in a nursery is also being evaluated. This virus, which is further attenuated by growth in tissue culture, is being developed by the Virus Research Institute, Boston. It is currently in phase II trials in infants at an NIAID Vaccine and Treatment Evaluation Unit (VTEU) and other sites. Two additional human viruses isolated from asymptomatic children have been isolated in India and will soon be evaluated in phase I studies in NIAID VTEUs for safety and immunogenicity. If they appear promising in these preliminary U.S. studies, they may be tested in India as potential vaccines for use in that country or region. One advantage that these weakened human viruses may have is the lack of vaccine-induced fever, a side effect seen in a small percentage of recipients of the rhesus- or bovine-based reassortant vaccines.

An NIAID grantee has succeeded in assembling virus-like particles from the products of bacculovirus-expressed rotavirus genes. The resultant particles are noninfectious and can be designed to contain structural proteins sharing multiple serotypes. This recombinant particle vaccine will be given parenterally, and the results obtained thus far in animals have been promising. It is hoped that safety studies soon will be conducted in humans. NIAID-supported research also is examining the possibility of incorporating rotavirus into microspheres for use as an oral vaccine capable of stimulating a protective mucosal immunity. This technique has been shown to enhance the immune response to either live or killed rotavirus administered either orally or parenterally.

Animal studies performed by an NIAID grantee have indicated that VP6 may be a new vaccine target. IgA monoclonal antibody directed against this protein induces protective immunity against rotavirus in mice. Studies by another grantee have indicated that a nonstructural protein, NSP-4, has secretory activity similar to that of enterotoxins. This is an interesting finding that may help explain the diarrheagenic activity of rotavirus infection. Whether induced immunity to this protein would be a useful vaccine strategy remains to be seen. Another NIAID grantee is testing the possibility of using DNA vaccines to induce protection against rotavirus in animals. The DNA vaccines, administered orally after the DNA was encapsulated in microspheres, were shown to be immunogenic and protective in mice. Studies of this nucleic acid vaccine approach are proceeding in pigs.

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Shigella

Shigellosis (bacillary dysentery) is endemic throughout the world. More than 32,000 cases were reported in the United States in 1993; this represents an increase of 35 percent from the number of cases reported in 1992. More than 90 percent of all cases reported in the United States were caused by *Shigella sonnei*. Symptoms of the disease can vary from mild diarrhea to severe dysentery with inflammation and ulcerative lesions of the colon, bloody diarrhea, and hemolytic uremic syndrome; death can result in untreated cases. Although there are 30 serotypes of shigellae, usually only two or three serotypes predominate in a given area. *S. sonnei* predominates in industrialized countries, whereas *S. flexneri* is most commonly found in developing countries; both are associated with endemic disease. *S. dysenteriae* causes epidemic outbreaks of dysentery, as well as significant endemic disease. Thus, a comprehensive vaccine approach to controlling shigellosis must include components of all three species.

Early studies showed that the O somatic antigens of *Shigella* are major immunogens and that the most effective attenuated vaccines are those that transport these immunogens to mucosal tissues, where they can generate a local or mucosal immune response. Limited tissue invasion of the vaccine strain would also likely generate a better cell-mediated immunity, thought to be important for protection against invasive pathogens such as *Shigella*.

Attenuated strains of *Shigella* have been created by deleting known virulence factors. Dr. Phillipe Sansonetti at the Pasteur Institute has made a *ics*A, *iuc*A deletion mutant of *S. flexneri* 2a (strain SC602). After a single oral dose of 10^4 cfu, this vaccine candidate provided 100 percent protection against severe shigellosis in North American volunteers when they were challenged with *S. flexneri* 2a.

Auxotrophic mutants also appear promising in animal studies, which indicate that they may be both sufficiently attenuated and still able to induce protective immunity. Researchers at the University of Maryland have created an *aro*A, *ics*A deletion mutant (strain CVD 1203) and a *gua*B-A, *vir*G deletion (CVD 1205) in *S. flexneri* 2a. Two doses of CVD 1203 proved safe at 10^6 cfu and induced IgA-secreting cell responses in 60 percent of volunteers. At higher doses (10^8 and 10^9 cfu) better antibody-secreting cell (ASC) responses resulted but at a price of increased vaccine reactogenicity. Additional deletions of two *S. flexneri* enterotoxins, SHET 1 and 2, from these strains should reduce reactogenicity and should be evaluated in humans in the near future.

Auxotrophic strains are also being developed as vectors for multivalent vaccines. An *S. flexneri aro*D deletion vector expressing *S. dysenteriae* 1 Shiga toxin B subunit has recently been reported. It should be noted, however, that even in persons infected with wild-type *S. dysenteriae*, an anti-Shiga toxin response has not been detected. Therefore, it is difficult to predict the protective efficacy that might be achieved by a vaccine strategy aimed at Shiga toxin. It is presumed that a humoral response directed against the toxin may lessen the chances of developing some of the more serious consequences of infection such as the hemolytic uremic syndrome.

Efforts also are under way in the laboratory of Dr. John Robbins at the National Institute of Child Health and Human Development to develop parenteral vaccines composed of detoxified *Shigella* lipopolysaccharide-protein conjugate. A randomized, double-blind study has been conducted in Israeli military volunteers and demonstrated 74 percent protection.

In studies conducted at the Walter Reed Army Institute of Research, two approaches for the development of *Shigella* vaccines are being examined. In one, subcellular nucleoprotein preparations have provided encouraging results in animal models of *S. sonnei* infection; in the other, a lipopolysaccharide-proteosome preparation specific for *S. sonnei* has also yielded promising animal results. Both approaches are being pursued as human vaccines by the Department of Defense.

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Typhoid

Typhoid fever remains a serious public health problem throughout the world, with an estimated incidence of 33 million cases and 500,000 deaths annually. It also is a serious threat to travelers visiting endemic areas. In the United States, more than 41,000 cases were reported in 1993. In virtually all endemic areas, the incidence of typhoid fever is highest in children from 5 to 19 years, which is important since school children can be immunized readily through school-based immunization programs.

Although licensed parenteral whole-cell vaccines are now available for typhoid fever, they are rarely used because they are only marginally effective and they induce adverse reactions in many vaccinees. Oral killed whole-cell preparations, though not reactogenic, are not protective against *Salmonella typhi*. Thus, efforts now center on the use of purified virulence (Vi) antigens (see below) or live orally administered preparations of demonstrable efficacy.

In collaboration with the Pasteur Institute, the National Institute of Child Health and Human Development has developed a nonreactogenic, immunogenic, purified Vi antigen vaccine; the Vi antigen is a linear homopolymer of galacturonic acid. Clinical trials in Nepal and South Africa demonstrated that a single injection has an efficacy of about 72 to 80 percent, in the face of a very high "force of infection." Since the Vi vaccine is effective after only one immunizing dose, it appears to offer some advantages over the Ty21a vaccine (see below), especially for use in developing countries, although it is associated with some minor side effects in some vaccinees. The Vi vaccine has been licensed in France and several countries in Africa; the manufacturer is currently assembling data to apply for a license in the United States. It has more recently been shown that immunogenicity could be increased by covalently conjugating the Vi polysaccharide to protein carriers such as *E. coli* labile toxin. The use of labile toxin or cholera toxin in such a vaccine may also stimulate antibody production to the toxin itself, thereby providing some protection against enterotoxigenic *E. coli*, cholera, and diarrheal diseases mediated by these related toxins.

An important advance for the control of typhoid fever has been the development of the attenuated *S. typhi* strain Ty21a from strain Ty2. This strain was extensively tested in Egypt and Chile, and although its efficacy may vary widely from site to site and with vaccine formulation, the Ty21a vaccine has been remarkably safe and reasonably immunogenic. It was licensed in the United States in 1991 and is presently being used primarily as a vaccine against traveler's diarrhea. The World Health Organization has recently advocated a head-to-head comparison of the efficacy of Ty21a and Vi to make future recommendations on the use of these two available vaccines in areas severely affected by typhoid.

Several groups of investigators have been developing attenuated deletion mutants as live oral vaccines. Metabolic pathways and genes critical to virulence expression have been targeted. These include the double *aro* mutants, *aro/pur* mutants, *cya/crp*, and the *pho*P / *pho*Q mutant. Several of these mutants have been in clinical trials with varying degrees of success. Focus here will be on recent efforts.

The University of Maryland has been pursuing double *aro* mutants derived from wild-type strain Ty2. CVD 908 was shown to be incompletely attenuated because it induced bacteremia in 6 of 12 volunteers at a dose of $5x10^7$ colony-forming units (cfu). The additional deletion of *htr*A made it clinically more acceptable. This strain, designated CVD 908-*htr*A, will soon be undergoing additional clinical studies. These vaccine strains are being developed by Peptide Therapeutics Limited, England.

Another vaccine candidate developed by Dr. Roy Curtiss is the *cya/crp/cdt* triple deletion mutant of Ty2. The *cya/crp* double mutant was found in clinical trials to be incompletely attenuated. Therefore, a portion of the gene adjacent to the *crp* locus was deleted. This gene was designated *cdt* since its apparent function is to control dissemination of *Salmonella* out of the intestinal tract and GALT to visceral organs in animals infected with *S. typhimurium* or *S. cholerasuis*. The strain of *S. typhi* containing equivalent deletions has been named x4073. This strain or derivatives thereof containing the balanced lethal plasmid expression vector have been used in two different clinical trials and shown to be well tolerated and immunogenic. Since most of the vaccine studies to date have employed strain Ty2 as the parent, and since this strain has been maintained in the laboratory since 1918 and probably contains a number of unknown mutations, Dr. Curtiss has made identical deletions in a recent clinical isolate in an attempt to define more clearly the genes contributing to attenuation as defined in mice and humans. The goal is to retain enhanced immunogenicity while satisfactorily attenuating the strain. Clinical trials with the first of these attenuated strains are expected to begin in 1998 at the NIAID-supported St. Louis University Vaccine and Treatment Evaluation Unit. Dr. Curtiss' vaccines are being developed by Megan Health, St. Louis.

The other strain being actively pursued as a vaccine against typhoid is the *pho*P / *pho*Q deletion mutant TY800. This strain has also employed Ty2 as the parent. The *pho*P / *pho*Q virulence regulon is a two-component system composed of a membrane-bound

kinase (PhoQ) and a cytoplasmic transcriptional regulator (PhoP). This system regulates a number of genes that contribute to *Salmonella* pathogenesis, and its deletion from Ty2 has created a vaccine candidate that appears to be well tolerated, even at a dose of $8x10^9$ cfu, and highly immunogenic. Of particular interest is the high antibody-secreting cell (ASC) response observed in volunteers to date. NIAID is hopeful that phase II trials with this strain can be conducted in the near future. The vaccine is being developed by Virus Research Institute, Boston.

Because *S. typhi* is an invasive organism, it is expected that significant cell-mediated immunity will be an important component of protection. Additionally, it is still presumed that *Salmonella* vectors can be developed to express foreign antigens and serve as multivalent vaccines capable of protecting against more than one enteric (or other) disease by oral immunization. Although encouraging results have been demonstrated in animals, this concept has yet to be conclusively demonstrated in human trials. Definition of a suitable live-attenuated, orally delivered vaccine against *S. typhi* itself will set the stage for this development.

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Fungal Infections

Overview

Infections caused by systemic fungal pathogens are a significant health problem in both the immunocompetent and the immunocompromised host. Fungi that regularly infect and cause disease in otherwise healthy hosts are termed primary pathogens. These include *Coccidioides immitis*, *Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Paracoccidioides brasiliensis*, and on occasion, *Cryptococcus neoformans*. Opportunistic fungal pathogens, which more typically require immunosuppression to infect the human host, include *Candida albicans*, which is a normal inhabitant of the human gut, and *Aspergillus fumigatus*, which is ubiquitous in the environment. The primary fungal pathogens each occupy a discrete ecological niche. *C. immitis* is found in the soils of the southwestern United States, Mexico, Central America, and South America. *H. capsulatum* can be found in soils enriched with guano from bats, chickens, and starlings, with a highly endemic focus along the Mississippi River but with documented occurrence throughout the world. *B. dermatitidis* is believed to be present in microfoci of soil worldwide, but chiefly in geographic regions of North America that overlap those of *H. capsulatum*. Historically, it has been difficult to isolate *B. dermatitidis* from the environment, but it probably occupies a different niche than *H. capsulatum*. Recent studies have found *B. dermatitidis* in moist, rich soil at the banks of rivers and waterways in endemic regions. *P. brasiliensis*, the etiologic agent of paracoccidioidomycosis (South American blastomycosis), is restricted to South and Central America, but its natural habitat remains elusive. *C. neoformans* can be found in soils contaminated with pigeon guano and is prevalent worldwide. Infection is initiated by inhalation of microscopic forms of each fungus from a point source in nature.

The true incidence of infection by these agents is difficult to assess because the diseases are not reported nationally and can be difficult to diagnose. With the exception of the latex agglutination test for cryptococcal capsular polysaccharide antigen, there are few widely available serologic tests to facilitate rapid laboratory identification of the systemic mycoses. Rather, definitive diagnosis relies on culture of the etiologic agent. Recent developments in molecular studies of *C. immitis*, which include cloning and expression of the diagnostic complement fixation (CF) antigen, and report of a sensitive polymerase chain reaction-based method for detecting coccidioidal DNA in patient sputum provide the basis for new clinical methods of rapid and inexpensive diagnosis of coccidioidomycosis.

It has been estimated, based on the results of skin tests, that there are between 25,000 and 100,000 new infections with *C. immitis* each year. The respiratory disease, known as Valley Fever, can occur in epidemic proportions; 1,500 seroconversions were documented in one county in California in 1991, whereas the number of

officially reported cases for the entire State was under 1,300. This finding underscores the problem of underreporting for these diseases. The epidemic in California resulted in more than 3,000 cases occurring in Kern County alone in 1992, and with the number of cases reported statewide in 1993 nearly equaling those reported statewide in 1992. It was estimated that the epidemic resulted in more than $45 million in medical costs in Kern County between 1991 and 1993. The California Department of Health sponsored a conference on coccidioidomycosis in 1993. The development of a vaccine was considered to be a promising approach for the prevention of the disease. The Valley Fever Research Foundation, a private foundation incorporated in 1993, commissioned a vaccine feasibility study. The study concluded that a vaccine effort should go forward.

The number of cases of Valley Fever in the Tucson and Phoenix areas increased by 66 percent between 1991 and 1992. A serious complication of the infection is meningitis, a life-threatening disease that is difficult to treat. Primary infections that apparently have resolved spontaneously may leave persistent but dormant fungal elements in lung tissue. Relapse of fungal diseases, such as Valley Fever, is viewed as a potential crisis among immunocompromised patients, such as those with acquired immunodeficiency syndrome (AIDS). One prospective study documented a prevalence of 25 percent in one cohort of HIV-infected patients over a 41-month period in highly endemic areas.

Histoplasmosis also is associated with epidemics in immunocompetent hosts; however, it is increasing significantly in immunocompromised hosts, such as those with AIDS, where the incidence of this fungal disease can be as high as 27 percent. The disease can resemble tuberculosis and has been misdiagnosed as such. In one study, 19 percent of patients with histoplasmosis had combined infection with tuberculosis. The disease is geographically widespread, with reports from every continent except Antarctica, and 500,000 new infections are estimated to occur annually in the United States. It is estimated that 99 percent of the infections resolve spontaneously, and the remaining 1 percent progresses to chronic or disseminated disease. The reasons for this progression in otherwise healthy individuals remain unknown. Additionally, clinical disease can be classified as mild, moderate, and severe, with the latter category being the most difficult to treat with available chemotherapy. Given the widespread distribution of disease, the inability to prevent acquisition from a point source in nature, and the remaining problems in antifungal therapy, a vaccine for this disease would have obvious public health benefits.

Blastomycosis occurs mainly as a sporadic infection in immunocompetent hosts, but many epidemics and cases of opportunistic infection among AIDS patients and other immunocompromised hosts have been described. The true incidence and prevalence of blastomycosis are unknown but seem to be lower than with the other systemic mycoses described here. A distinguishing feature is the high frequency of clinical disease versus mild and asymptomatic infections among infected persons, highlighting the organism's pathogenicity. Another feature is that blastomycosis is a common infection in dogs that reside in endemic zones. The severity and lethality of most canine infections underscore the potential of *B. dermatitidis* as a primary pathogen.

Although immunosuppressive therapy and infection with HIV are recognized risk factors for the development of severe, progressive coccidioidomycosis and histoplasmosis, they are not prerequisite to human infection with the causative fungi. Both are primary pathogens. In addition, subclinical infection with these fungi and with *C. neoformans* poses a threat of subsequent reactivation to a progressive form of disease with the advent of immunosuppression. Cryptococcosis (cryptococcal meningitis) is a worldwide problem for immunosuppressed patients. In the United States cryptococcosis is a well-known AIDS-defining illness and occurs in 7 to 11 percent of patients with AIDS. A hospital survey in New York City documented more than 1,200 cases of cryptococcosis in 1991 that were primarily associated with HIV-infected patients, resulting in a yearly prevalence of 6 to 8 percent in this population. Cryptococcal meningitis is also prevalent in HIV-infected individuals in Africa where the costs of antifungal therapy can be prohibitive. Even with the advent of newer antifungal drugs, such as the triazoles, treatment remains suboptimal, and no existing treatment is curative. The same is true for coccidioidomycosis and histoplasmosis in patients with AIDS.

Mechanisms of virulence for the pathogenic fungi are poorly defined. The fungi considered above lack toxins that could serve as good targets for a rationally designed vaccine; furthermore, they possess a complex, eukaryotic genome that makes elucidation of their molecular biology more difficult than that for either their viral or bacterial counterparts. Nonetheless, fungi present numerous effective antigens as demonstrated by the host's response to infection. In general, cell-mediated immunity is thought to be more important to recovery from infection than the antibody response. One possible exception is cryptococcosis, in which antibody specific for the capsular polysaccharide has an opsonizing effect on the encapsulated fungus. With an ever-expanding immunocompromised host population at risk for all of these fungal infections, and with the inability of even new antifungal agents to eradicate fungi from infected patients, one must give serious consideration to the preventive or therapeutic role of antifungal vaccines.

Source

Dixon DM, Casadevall A, Klein B, Mendoza L, Travassos L, Deepe GS Jr. Development of vaccines and their use in prevention of fungal infections. *J Med Vet Mycol.* Submitted.

Blastomycosis

Spores are inhaled into the lungs and convert into budding yeasts that are large and relatively resistant to phagocytosis and killing by the neutrophils and mononuclear effector cells that comprise the early inflammatory response. Within several weeks after infection of humans and experimental animals, the host develops acquired immunity to *B. dermatitidis*, as evidenced by the appearance of delayed-type hypersensitivity, proliferation of lymphocytes *in vitro*, and circulating antibodies in response to antigens of the fungus. In a murine model of blastomycosis, passively transferred T lymphocytes but not serum from immune to naive animals conferred protection, suggesting that immunity resides chiefly with antigen-specific T cells.

A 120 kD protein, designated WI-1, is displayed on the surface of *B. dermatitidis* yeasts and is an immunodominant antigen during human, canine, and experimental murine infection. Human

patients develop strong antibody and T lymphocyte responses to determinants of WI-1. WI-1 has been cloned and sequenced and shown to contain 30 copies of a repetitive domain of 25 amino acids similar in sequence to a bacterial adhesin, invasin. This so-called tandem repeat mediates binding of the yeast to integrin receptors on human cells, and the expression of WI-1 is altered on genetically related strains of *B. dermatitidis* that differ in virulence for mice. These observations suggest that WI-1 plays a key role in the pathogenesis of blastomycosis. Human, murine, and canine infection is associated with the development of high antibody titers directed against the tandem repeat, but the functional role of these antibodies *in vivo* is unknown. Bulk and clonal populations of T lymphocytes from human blastomycosis patients respond strongly to WI-1 in *in vitro* assays. At the clonal level, these cells are directed chiefly toward epitopes displayed in a short segment of amino acids at the N-terminus, but not at all to repetitive regions of the molecule. WI-1 is highly immunogenic in mice, where protective efficacy has been shown. These studies will define the potential role of WI-1 as candidate for vaccine development. A detailed understanding of B and T cell epitopes of WI-1 is an advantage that will permit flexibility and a fundamental investigation of different formulations of recombinant fungal vaccines. For example, the ability to vary the number and type of epitopes displayed will delineate the importance of B and T cell responses alone and together in providing optimal protection. The frequency and severity of canine blastomycosis offer the unique opportunity to undertake field trials of these novel vaccine formulations, such as in naked DNA vaccines, that might not otherwise be possible in humans.

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Hogan LH, Josvai S, Klein BS. Genomic cloning, characterization, and functional analysis of the major surface adhesin WI-1 on *Blastomyces dermatitidis* yeasts. *J Biol Chem* 1995; 270:30725-30732.

Candidiasis

Candidiasis is a leading group of opportunistic mycoses caused by any of several species of the genus, *Candida.* Most noteworthy examples include *C. albicans, C. tropicalis,* and *C. krusei.* These and other *Candida* species are normal inhabitants of humans and normally live in harmony with the mammalian host. Factors predisposing to disease include chemical immunosuppression, surgical trauma, and underlying diseases such as diabetes and AIDS. Neutropenia is a major risk factor, and patients undergoing immunosuppression for prevention of rejection of bone marrow or organ transplantation are particularly vulnerable for infection from either endogenous or exogenous sources.

Novel advances in the identification of protective antibody in models of cryptococcosis described in this report have given hope that analogous situations may pertain to other opportunistic mycoses, including candidiasis. Indeed, a protective antibody has been identified for *C. albicans* in an animal model system. Antigen delivery was key to demonstrating that a mannan adhesin from the fungus could generate immunoprotection. Liposome encapsulation of a mannan adhesin fraction of yeast cells has been used to generate protective antibodies that are functional in vaccinated mice and were passively transferred to protect normal and immunocompromised mice. Both protective and nonprotective antibodies were identified. The latter can be useful in addressing the controversy generated in previous studies where circulating antibody did not correlate with protection. A murine monoclonal antibody, B6.1, has been demonstrated to be protective in passive transfer experiments, and substantial interest has been kindled in examining the role of immunotherapy as an alternative to chemotherapy in human candidiasis. Given the newly acknowledged problem of antifungal drug resistance in *Candida,* these findings are of special relevance.

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Coccidioidomycosis

Spores of *C. immitis* are inhaled into the lungs, where they undergo a morphological conversion to a parasitic, spherule form of growth. The spherule enlarges and subdivides into propagative units that are released to repeat the cycle. Patients develop delayed-type hypersensitivity as a consequence of infection. Although complement-fixing and precipitating antibodies are produced during the course of infection, they do not seem to be protective. Indeed, high titers of complement-fixing antibodies are a poor prognostic sign. In experimental infections, immunity is transferred by thymus-derived lymphocytes (T cells) but not by serum.

An experimental vaccine has been prepared from formalin-killed spherules of the fungus grown *in vitro.* After demonstrating that the vaccine reduced the percentages of animals dying from experimental infection, a trial was conducted in human volunteers. The study groups were from Arizona and California and were demonstrated to be skin test-negative to spherule antigen and to coccidioidin prior to vaccination. A total of 1,400 subjects received the formalin-killed spherulin vaccine (1.75 mg per injection, with a total of three injections), and 1,400 others received placebo. The results of the trial indicate that the vaccine did not prevent clinically apparent coccidioidomycosis. In experimental trials in mice, the vaccine did not prevent infection but did prevent progressive disease and death. Because progressive disease did not occur in either the control or vaccine-administered human groups, it was not possible to evaluate these potential protective effects. Failure of this trial could have been due to dose-limiting irritation at the injection site from nonessential cell-wall polysaccharides in the vaccine that prevented effective immunization by critical proteins. This possibility can be tested as the protein antigen(s) responsible for protection are identified and used in a more purified preparation or as a recombinant vaccine. Disruption of the whole spherule vaccine and centrifugation at 27,000x gravity have produced a supernatant preparation with demonstrable murine protection. One antigen identified in the supernatant is a glycoprotein. After deglycosylation, reverse phase high performance liquid chromatography elution profiles indicate that this protein is identical to a 33 kD protein purified from the cell wall of

mature spherules. The gene coding for this protein is now being cloned from a cDNA library, and the recombinant product could be tested.

Additional vaccine-related research is under way with various fractions of this fungus. Attempts are being made to isolate and characterize T cell-reactive molecules and to clone the relevant genes for large-scale expression of those molecules capable of mediating protective immunity. Candidate molecules that elicit T cell-mediated immune responses were originally identified by T cell immunoblot assays of fusion proteins expressed in cDNA libraries of *C. immitis.* A 200-base pair cDNA was isolated from an expression library by an immunological screen with antibody raised against a crude T cell-reactive fraction. The cDNA encodes a 7.3 kD recombinant protein that was shown to be highly immunoreactive in a T cell proliferation assay. The full-length gene was subsequently isolated, shown to encode a 45.2 kD protein with 70 percent homology to mammalian 4-hydroxyphenylpyruvate dioxygenase (4-HPPD), and expressed in a bacterial vector. Both the purified recombinant protein and the *C. immitis* gene subcloned into a naked mammalian expression vector (pcDNA3) have been used as immunogens and have been evaluated for their ability to immunoprotect mice against coccidioidal challenge by the intraperitoneal and intranasal routes. Cytokine production resulting from immunization with the recombinant protein has been examined. A heat shock protein (HSP60) is currently being evaluated in this same manner. This systematic approach is used to identify a multiplicity of antigens that elicit T cell-mediated immune response to coccidioidal infection and could be incorporated into a vaccine effort.

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Cryptococcosis

Yeast cells of *C. neoformans* are thought to be the infectious form of the fungus. Inhalation of these cells establishes a primary pulmonary infection that is often nonapparent. Meningitis is the typical manifestation of disease. Early diagnosis and treatment can arrest but not cure infection in AIDS patients; lifetime suppressive therapy is required.

C. neoformans is delimited by a polysaccharide capsule and, therefore, is unique among the major fungal pathogens of humans. The antibody response to the capsular polysaccharide is minimal in clinically apparent infections. Since most patients with cryptococcal meningoencephalitis have soluble capsular polysaccharide in serum or cerebrospinal fluid, testing for antigen is useful in the diagnosis of this infection. The capsule of *C. neoformans* is a known virulence factor, and attempts have been made to induce a protective immune response against capsular polysaccharide. Injection of mice with capsular polysaccharide alone or with adjuvants does not appear to result in sustained or high-titer antibody response. However, conjugation of cryptococcal polysaccharide to protein carriers may provide for an improved antibody response. Cryptococcal glucuronoxylomannan conjugated to tetanus toxoid has been shown to be immunogenic in mice. Preliminary clinical trials with a glycoconjugate vaccine have been conducted to determine safety and antigenicity; the ultimate goal is to develop a vaccine that will protect patients at high risk of developing cryptococcosis.

Antibody administration has been shown to enhance the efficacy of amphotericin B, fluconazole, and 5-fluorocytosine in mouse models of infection. Studies of antibody efficacy in mice have shown that antibody specificity and isotype are important characteristics for antibody efficacy. Vaccines that elicit primarily protective antibodies may be effective in preventing infection even if the role of naturally occurring antibody in protection is uncertain.

Confirmation of the protective role of antibody also comes from studies showing that the infusion of monoclonal antibody can prolong life and decrease fungal burden in mice challenged with fungi by the intraperitoneal, intravenous, or intracranial routes. Several protective murine monoclonal antibodies have been used to construct mouse-human chimeric antibodies to the cryptococcal polysaccharide; the goal of clinical studies, in this case, is to determine the efficacy of passive immunization as an adjunct to chemotherapy in cryptococcal meningitis.

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Histoplasmosis

Spores of *H. capsulatum* are inhaled into the lungs and convert into budding yeasts that proliferate within cells of the macrophage lineage. The importance of T cell-mediated immunity in infection is implicit in the emergence of this fungus as a significant pathogen in AIDS. As for coccidioidomycosis, antibodies can be diagnostic but are not thought to play a major protective role. Delayed-type hypersensitivity develops, and immunity can be demonstrated following transfer of T cells in experimental models. These models have shown the expansion of both suppressor and helper cell lines in response to challenge with fungal antigens. The recent development of a transformation system for *H. capsulatum* and an increased knowledge of its molecular biology should facilitate studies on pathogenesis and virulence and provide at least the methodological basis for vaccine development.

HIS-62 is a 62 kD glycoprotein antigen isolated from cell wall and cell membrane extracts of yeast cells of *H. capsulatum*. This antigen induces cell-mediated immune responses in C57BL/6, BALB/c, and CBA/J mice. Vaccination with 80 micrograms of HIS-62 significantly protects all three strains of mice against lethal challenge with viable cells of the fungus. In addition, lymphocytes from humans exposed to *H. capsulatum* respond *in vitro* to this antigen. The gene encoding this antigen has been cloned and sequenced; it has a high homology with the gene that encodes for heat shock protein 60 (HSP60). Recombinant antigen has been generated from *E. coli*, and it stimulates monoclonal populations of antigen-reactive T cells and polyclonal T cells from mice immunized with *H. capsulatum* yeast cells. Vaccination with the recombinant antigen protects mice against pulmonary histoplasmosis. Current efforts are focused on mapping the epitope or epitopes that may be involved in conferring protection. Preliminary results indicate that it is possible to isolate protective domains from within the full-length gene, but initial studies have found the protective fragments to be less effective in immunoprotection than the whole protein. These studies continue, as do studies with other recombinant proteins.

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Paracoccidioidomycosis

Natural infection with *Paracoccidioides brasiliensis* is presumed to be via the respiratory route. The disease may disseminate to chronic progressive forms that are characterized by a vigorous cellular immune response. Depression of cellular immunity may occur over the course of disease and may be reversed. Antibody titers typically rise but do not confer protection in natural infection. Given the similarities between paracoccidioidomycosis and both coccidioidomycosis and blastomycosis, it would be predicted that native antigens exist that can be used to generate a protective immune response. Investigations are under way that support this prediction. Most actively studied is a 43 kD antigen (gp 43) from yeast cell cultures. It represents the major diagnostic antigen and is immunodominant. The gene for gp 43 has been cloned and sequenced, and an immunodominant, 15 aa residue has been identified. Mice immunized with either gp 43 or the 15 aa protein have been shown to have significant reductions in colony-forming units in the lungs following intratracheal challenge with *P. brasiliensis*.

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Pythiosis

Phthium insidiosum is a filamentous eukaryotic organism previously classified in the Oomycetes of Kingdom Fungi, but recently moved to Kingdom Chromista (Protoctista). The organism is aquatic and has a flagellated stage. Cutaneous, subcutaneous, and systemic disease can result in humans, horses, and other animals as a consequence of traumatic implantation. Mortality rates of 60 percent occur with untreated individuals. Choices of chemotherapy are limited, and antifungal drugs are generally not effective. At least two different groups of investigators have generated promising results with therapeutic vaccines consisting of hyphal extracts. Rates of 53 percent efficacy have been reported following injections of such extracts in infected horses. Refinement of extracts by supplementation with purified protein derivatives has increased efficacy to as much as 70 percent with chronic pythiosis, which is least responsive to treatment. Three immunodominant proteins (28, 31, and 32) have been identified. A single case of human vaccination has been described for a young Thai boy with phthiosis refractory to multiple courses of antifungal therapy. The infection resolved following vaccination. Research with this novel therapeutic vaccine approach for a fungus-like organism is ongoing.

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Herpesvirus Infections

Overview

The eight human herpesviruses—which include herpes simplex viruses (HSV) types 1 and 2, Epstein-Barr virus (EBV), cytomegalovirus (CMV), varicella-zoster virus (VZV), and human herpesviruses -6, -7, and -8 (HHV-6, -7, and -8)—are a significant public health problem in the United States. Most of the population has been infected with several of these herpesviruses and thus has life-long latent infections.

Primary infections are not usually severe or life-threatening in healthy individuals, but many of the human herpesviruses can produce severe or chronic active infections in certain individuals. Whereas primary infection of young children with most herpesviruses is often unrecognized or mild, primary infection in adults with VZV or EBV can be severe. HSV and CMV pose a particular threat to newborns whose mothers had a primary infection during pregnancy. Herpesvirus infection can also have long-term consequences. In certain geographical areas and populations, EBV is associated with nasopharyngeal carcinoma and with Burkitt's lymphoma. More recently, the association of EBV with Hodgkin's lymphoma, T cell lymphomas, and some gastric carcinomas has been suggested. HHV-8 is a newly recognized herpesvirus that appears to be associated with Kaposi's sarcoma.

Reactivation-associated disease is often more severe than primary infection. HSV-1, HSV-2, and VZV are associated in some individuals with frequent and/or painful recurrences, which manifest themselves as cold sores, genital herpes, and shingles, respectively. Reactivation of herpesviruses in individuals with compromised or waning immunity may result in severe and life-threatening illnesses such as CMV pneumonia and EBV-associated lymphomas. Thus, herpesviruses can pose a particular threat to AIDS patients, cancer patients, organ transplant recipients, and the elderly. Induction of immunity that can withstand immunosuppressive regimens would bring significant benefit to these patients. An additional concern with reactivation is that asymptomatic individuals shedding reactivated virus may serve as reservoirs for herpesvirus transmission.

The correlates of protective immunity for herpesvirus infections are not fully understood. Although there is substantial evidence for the importance of both cellular and humoral immune responses directed against specific viral glycoproteins, a number of factors complicate the design of herpesvirus vaccines. For example, virus-specific immunity appears to be important in preventing reactivation of latent virus, but in many cases reactivation can occur even in the presence of humoral and cell-mediated immunity. In addition, most human herpesviruses infect via the mucosal surfaces; immunity at these sites is not well understood. A further complication is the immune evasion strategies that most herpesviruses have developed. The role of these processes in modulating the level of vaccine-induced immunity (for live vaccines) or in blocking the vaccine-induced immune response to a challenge infection is not well understood.

Several live and subunit vaccines have been developed and evaluated for efficacy in preventing herpesvirus infections. The only herpesvirus vaccine presently licensed by the Food and Drug Administration is the live-attenuated Oka strain of VZV. A concern with live vaccines for herpesviruses is that latency can be established and that there is thus the potential for reactivation-associated disease. However, extensive studies of the Oka vaccine in healthy and leukemic children, as well as the attenuated Towne strain of CMV in renal transplant recipients, demonstrate that under appropriate circumstances attenuated virus vaccines can be used successfully, even in immunocompromised individuals. Efforts are under way to engineer new attenuated vaccines by identifying and manipulating viral genes that control latency, reactivation, and virulence.

Subunit vaccines containing purified viral proteins are a possible alternative to live vaccines. Most studies have focused on the external viral glycoproteins; however, early viral antigens also have been shown to induce T cell-mediated immunity. Furthermore, the host's response to some viral antigens has been shown to be HLA-restricted. All of these factors certainly will have to be addressed in the design of subunit vaccines. To date, experience with subunit vaccines for herpesviruses has not been encouraging. For example, although subunit vaccines consisting of one or more purified HSV proteins can elicit both humoral and cellular immune responses, a recent phase III trial of an HSV-2 gB+gD subunit vaccine failed to prevent or delay outbreaks in infected individuals. New approaches for delivery or presentation of viral antigens (e.g., DNA immunization) may be required to produce adequate protection from subunit vaccines.

Cytomegalovirus

Background

Approximately 50 percent of the U.S. population is seropositive for CMV. Seropositivity varies with socioeconomic status and geographic location: 40 to 60 percent in middle-income groups; up to 80 percent in lower socioeconomic groups. The outcome of CMV infection is highly dependent on the immune status of the host. Primary infection in healthy individuals is likely to be asymptomatic or may cause a mild mononucleosis-like syndrome. However, in patients with deficient or immature immune systems, CMV infection can be a serious, even life-threatening problem.

Congenital CMV is the most common intrauterine infection in the United States, occurring in 0.4 to 2.3 percent of all infants born alive. It is estimated that 37,000 to 40,000 infants in the United States are born with congenital CMV each year. About 3,000 to 4,000 infected newborn infants per year have symptomatic CMV disease; of those who survive, most suffer from profound progressive deafness and/or mental retardation. An additional 4,500 to 6,000 children who are asymptomatic at birth also develop serious handicaps. The highest risk for congenital CMV infection is among infants born to mothers who have had primary infection during pregnancy. In the United States, congenital CMV may be the cause of 20 to 40 percent of congenital deafness and is as frequent a cause of mental retardation as the fragile X-chromosome. The cost of custodial care for severely damaged children in the United States is estimated at $1.86 billion annually.

Organ Transplants. CMV is the single most important infectious agent affecting recipients of organ transplants, with at least two-thirds of these patients developing CMV infection or reactivation 1 to 4 months after transplantation. Also, about 15 percent of bone marrow transplant recipients develop CMV pneumonia;

without treatment, such infections are fatal about 80 percent of the time. Although less severe, active CMV infection occurs in 20 to 60 percent of all liver transplant recipients.

AIDS-Associated Infection. CMV causes five distinct neurological syndromes in patients with AIDS. CMV retinitis occurs in 6 to 15 percent of AIDS patients, and it is estimated that CMV enterocolitis occurs in at least 2.5 percent of AIDS patients.

Current Status and Key Issues in Research and Development

Although the correlates of CMV immunity are not precisely known, clinical observations suggest that preexisting humoral and/or cellular immunity may reduce the severity of disease. Maternal antibody in seropositive women appears to significantly reduce both the incidence and severity of congenital infection, and passive immunoglobulin therapy may benefit some transplant recipients. In addition, infusion of *ex vivo* expanded CMV-specific cytotoxic T lymphocytes (CTL) appears to reconstitute immunity and provide protection against disease in bone marrow transplant recipients. The major CMV immunogenic protein appears to be the surface glycoprotein gB. This protein induces the development of both virus neutralizing antibodies and T cell-mediated immunity, and the T helper cell response to gB is HLA class II restricted. The viral tegument protein (pp65, from the UL83 gene) has been shown to be a major target for $CD8^+$ CTLs during natural infections. Other viral antigens, including the surface glycoprotein gH and additional early antigens, also are being considered for use in vaccines. Despite the presence of gB neutralizing antibodies, virus can be reactivated and infections caused by other strains of CMV can occur; indeed, multiple strains of CMV have been identified. An additional concern in vaccine design is that CMV employs several strategies that prevent the host immune system from recognizing infected cells and that could potentially interfere with the ability of a live-attenuated vaccine to stimulate a protective cellular immune response.

Several CMV vaccination strategies have been evaluated in humans. A live-attenuated strain (Towne) stimulates both humoral and cellular immunity, although less than natural infection. The efficacy of Towne has been evaluated in several clinical studies: Protection has been documented in seronegative women and transplant recipients but is less than that afforded by a natural infection, and complete protection has been achieved against only low doses of challenge virus. Further efforts are needed to improve the immunogenicity of live-attenuated vaccines (see below for the approach taken by Aviron). Subunit vaccines have been shown to induce both humoral and cellular immune responses, but have not to date been able to prevent infection or disease. Evaluations of alternative vaccine formulations and antigens are under way. A subunit vaccine produced by Chiron Vaccines (Emeryville, California), consisting of recombinant gB (produced in CHO cells) and the adjuvant MF59, has been evaluated in phase I and II trials. The vaccine is well tolerated and highly immunogenic in seronegative adults and toddlers, and stimulates high levels of neutralizing antibody that cross-neutralize clinical isolates. Additional approaches are being evaluated in animal models. Delivery of gB via a canarypox vector has been tested in guinea pigs, and is capable of inducing both humoral and cell-mediated responses. DNA immunization holds out the promise of improving the presentation of individual viral proteins to the host immune system. Immunization with DNA plasmids encoding gB and the matrix protein pp65 has been evaluated in mice and induces both neutralizing antibody and CTL responses.

Recent Accomplishments and Developments

Engineering an improved live-attenuated cytomegalovirus vaccine. As noted above, the attenuated vaccine strain of CMV (Towne), while immunogenic, did not stimulate as high a level of immunity as that produced in a natural infection. Investigators at Aviron (Mountainview, California) are attempting to make Towne more immunogenic by replacing selected parts of its genome with sequences from nonattenuated strains of CMV. They have identified numerous differences between the genome of the Towne strain and that of wild-type CMV, including a large DNA segment present in the genomes of a virulent laboratory strain (Toledo) and of five clinical isolates, but not in the Towne genome. The extensive variation in genome sequence observed between these strains may explain the differences that they exhibit in virulence and tissue tropism. The investigators are now using this information, in conjunction with a unique method they developed to engineer changes in the CMV genome, to construct hybrid viruses that replace defined portions of the Towne genome with corresponding segments of a nonattenuated strain of CMV. Initial vaccine candidates have been created, and Aviron plans to initiate a phase I clinical trial in 1998 using a chimeric vaccine candidate.

CMV employs multiple mechanisms to evade cell-mediated immune responses. For a viral vaccine to stimulate a cell-mediated immune response, viral proteins must be broken down into peptides, which are then transported into the endoplasmic reticulum and displayed on the surface of the infected cell in conjunction with major histocompatibility complex (MHC) molecules. Multiple strategies employed by CMV to subvert this process could interfere with the ability of a live-attenuated vaccine to induce a protective cell-mediated immune response. Recent work has dissected out the mechanisms by which at least three CMV proteins act to interfere with the processing and MHC class I-associated presentation of viral peptides. One approach used by CMV is to downregulate expression of class I MHC molecules by facilitating the degradation of newly synthesized class I heavy chains. Hidde Ploegh and coworkers have shown that CMV expresses at least two genes—US11 and US2—which encode a product that causes the dislocation of newly synthesized class I heavy chains from the lumen of the endoplasmic reticulum to the cytosol. The US11 and US2 gene products have different specificities for class I molecules, suggesting that CMV has responded to the polymorphism of the MHC by evolving a diversity of functions that interfere with class I-restricted antigen presentation. A second point in the MHC/peptide presentation process is targeted by the product of the US6 gene. This glycoprotein has been shown to bind the transporter associated with antigen processing (TAP)-dependent translocation of peptide from the cytosol to the endoplasmic reticulum. The importance of these proteins in modulating the cell-mediated immune response to a live CMV vaccine remains to be determined.

Maintenance and reactivation of latent cytomegalovirus. Following initial infection, CMV remains latent in the host and under conditions of immune suppression—such as organ or bone marrow transplantation—can reactivate and produce significant

disease. Knowledge of the mechanisms of maintenance and reactivation of latent infection is important to developing vaccines that protect against reactivation disease and that do not contribute to such disease themselves. Recent studies have shed new light on several important aspects of CMV latency. Edward Mocarski and colleagues have characterized latent CMV transcripts in human granulocyte-macrophage progenitors. Both sense and antisense transcripts with the potential to encode small proteins are expressed in culture and in bone marrow aspirates from seropositive individuals. Antibodies reactive with two of these potential gene products are also detected in seropositive individuals. Overall, these results suggest that bone marrow-derived myeloid progenitors are an important natural site of viral latency. These cells are also the source of circulating monocyte-derived macrophages (MDM). Jay Nelson and colleagues have shown that allogeneic stimulation (similar to what would occur during a transplant) is required for productive CMV infection in these cells. They have also used allogeneic stimulation to show for the first time that latent virus can be reactivated from MDM isolated from seropositive individuals. Monocytes are therefore also a natural site of CMV latency from which the virus can be reactivated under conditions of allogeneic stimulation.

Next Steps/Challenges Ahead/What's on the Horizon

Further work is needed to define more precisely the key antigens and epitopes important for protection against infection, primary disease, and reactivation. The role of immune evasion in the induction and response to host immunity needs to be clarified. Several new vaccine candidates are in the initial stages of evaluation. Aviron is expected to begin phase I testing of its engineered live-attenuated vaccine in 1998. Chiron is extending its phase I studies of the gB/MF59 subunit vaccine. Clinical testing of canarypox-vectored and DNA vaccines is also on the horizon.

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Varicella-Zoster Virus

Background

Primary infection with VZV is manifested as chickenpox (varicella) and results in a life-long latent infection. Reactivation of the latent virus leads to shingles (zoster).

Varicella. Before the introduction of the live-attenuated vaccine (see below), approximately 4 million cases of varicella (chickenpox) occurred annually, primarily in young children, with more than 90 percent of the U.S. population becoming seropositive. Chickenpox was estimated to cost about $400 million each year, much of this representing the cost to parents of lost income from

work. As the use of the vaccine expands, it will lead to changes in the epidemiology and costs of this childhood illness in the United States.

Varicella can be complicated by a variety of serious conditions, including skin infections that can progress to systemic infections, infections of the brain, and pneumonia. Complications of varicella have been responsible for approximately 9,300 hospitalizations and 100 deaths annually. The risk of these complications is highest in adults: While less than 5 percent of varicella cases occur in adults over 20 years of age, 55 percent of the deaths occur in this age group.

Zoster. Zoster typically involves large areas of skin that ulcerate and require several weeks to heal. The skin eruption itself is very painful and is often followed by postherpetic neuralgia (PHN), a pain syndrome that persists for many months or years and that can be very disabling. There is no established prophylaxis or therapy for PHN. The incidence rate and severity of zoster and its complications increase with increasing age. The rate in 50-year-olds appears to be between 2 and 4 cases per 1,000 persons per year, and it more than doubles by age 80. More than one-half of all cases occur in persons 60 years of age and older. PHN is the major complication of herpes zoster in the immunocompetent host; rare in individuals below 40 years of age, PHN is estimated to occur in 25 percent to more than 50 percent of patients over 59 years of age.

Current Status and Key Issues in Research and Development

Both humoral and cellular immune responses are elicited early in primary VZV infections, and their relative contribution to protection from disease is not well understood. The impact of active humoral immunity appears to be limited, but preexisting antibody has been shown to provide some level of protection. Passively acquired maternal antibody affords some protection to infants, and post-exposure administration of VZV immunoglobulin (VZIG) to immunocompromised children reduces disease severity. Among children receiving the live-attenuated Oka vaccine, the incidence and severity of breakthrough infection are inversely correlated with antibody titer to VZV glycoproteins, and possibly with the level of T cell responses as well. Conversely, it is clear that cellular responses play the primary role in preventing disease associated with reactivation of latent VZV. Although decreases in humoral immunity are not associated with increased risk of zoster, the age-related decline in cell-mediated responses to VZV antigens is proportional to the age-related increase in the incidence and severity of zoster, suggesting that this loss is a causative factor. The role of viral immune evasion mechanisms in VZV infection is not well defined. For example, VZV is similar to HSV in that its glycoprotein gE forms a complex with gI and can act as an Fc receptor, but it is not known whether the similarity to HSV extends to providing protection from virus-specific antibody.

A live-attenuated varicella vaccine, Oka, was developed in Japan in the early 1970s. In the United States, this vaccine (Varivax) is produced by Merck & Co., was licensed for use in healthy individuals by the Food and Drug Administration in 1995, and is now recommended for universal use in early childhood by the CDC's Advisory Committee for Immunization Practices, the American Academy of Pediatrics, and the American Academy of Family Physicians. The use of Varivax in the United States has been increasing steadily. Merck & Co. has distributed more than 6 million doses and estimates that the private sector immunization rate for 1- to 2-year-olds is approaching 70 percent, while in the public sector all but three States have ordered the vaccine for use in their immunization programs. Post-licensure surveillance in day care centers indicates that the vaccine is generally well tolerated and demonstrates protective efficacy. The expanding use of this vaccine will undoubtedly alter the epidemiology and costs of varicella in the United States and affords the opportunity to study in greater detail the correlates of protection against infection and disease and the viral functions associated with virulence and attenuation. Long-term monitoring of vaccinees will establish the extent to which immunity persists and whether boosting will be required to maintain protection through adulthood. It also remains to be demonstrated whether the vaccine will be effective in other populations, such as in the elderly for prevention of zoster, or in immunosuppressed transplant patients. Initial studies of vaccination in the elderly have shown that VZV-specific cell-mediated immunity can be boosted significantly. In addition to further studies on the live-attenuated virus, there are continuing efforts to evaluate alternate vaccines, including subunits and naked DNA. Further studies are required to define the specific viral antigens that should comprise any subunit vaccine.

Recent Accomplishments and Developments

Varicella-zoster virus expresses immediate early and early genes during latent infection. Following primary infection, VZV establishes a latent infection in the host's dorsal root ganglia. Subsequent reactivation of the virus leads to zoster (shingles). While both viral functions and the host's immune response play important roles in the maintenance and reactivation of latent virus, little is known about the specific mechanisms. Recently, Anne Gershon and Saul Silverstein at Columbia University and Donald Gilden at the University of Colorado have shown that during latent infection VZV expresses up to four proteins that are also expressed during productive infection. This is unlike most other herpesviruses, which typically produce a unique set of transcripts during latency. It does appear, however, that in latently infected human ganglia these proteins are confined to the cytoplasm and that blocking entry of these proteins into the nucleus may be important to preventing virus replication. Further, Ann Arvin and colleagues have shown that infected individuals mount both humoral and cellular immune responses to one of these proteins, the product of the ORF63 gene. Further work is needed to determine whether these responses play an important role in preventing reactivation of the latent virus. Overall, these results advance the understanding how the virus and the host immune response control latent infection and may lead to the development of improved vaccines for preventing VZV reactivation and zoster.

Next Steps/Challenges Ahead/What's on the Horizon

The development of a VZV virus incapable of becoming reactivated, or of a subunit vaccine, will require much more basic research. Studies on the antigenic components most important for the development of an immune response in humans, and on novel methods for presenting viral antigens to cells of the immune system, are in progress.

A large phase III study (Veterans Administration Cooperative Study Program) has been designed to evaluate whether boosting VZV cell-mediated immunity in elderly persons will reduce the incidence of zoster or its complications. Preliminary dose-ranging studies are under way, and the full trial is expected to commence in 1998. Other populations at risk for severe VZV disease—e.g., pediatric renal transplant recipients—are also candidates for studies evaluating the safety and efficacy of the live-attenuated vaccine.

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Epstein-Barr Virus

Background

Based on serology, approximately 90 percent of the adult U.S. population has been infected with EBV. Primary childhood infection is often asymptomatic. In most developed countries, 35 to 75 percent of the young adult population remains seronegative. In 25 to 70 percent of such seronegative young adults, EBV infection results in infectious mononucleosis. In limited geographical areas and populations, EBV is associated with nasopharyngeal carcinoma (NPC) and with Burkitt's lymphoma (BL). NPC and BL appear to require environmental, genetic, or chemical cofactors. In immunocompromised individuals, including AIDS patients, EBV is associated with lymphoproliferative diseases and lymphomas. Recent evidence also suggests an association with Hodgkin's lymphoma, T cell lymphomas, and some gastric carcinomas.

Current Status and Key Issues in Research and Development

The principal target of EBV neutralizing antibodies is the major virus surface glycoprotein gp220/350. Several vaccine candidates based on this glycoprotein have been developed. For subunit vaccination, this large, heavily glycosylated protein has been prepared

from mammalian cell lines (Chinese hamster ovary or mouse C127). Primate studies demonstrate that subunit vaccination can elicit a specific antibody response that is at least partially protective and suggest that the choice of adjuvant is likely to be important in achieving acceptable efficacy. Live recombinant vectors have also been used to express and deliver gp220/350. Immunization with vaccinia recombinants provides some protection in primates and in EBV-negative infants. A range of cell-mediated responses to EBV infection have also been described and are likely to be important in controlling persistent infection. CTLs specific for the latent EBV nuclear antigens EBNA-3A, -3B and -3C are predominant in a large portion of seropositive adults and children. Clinical trials of a peptide vaccine bearing an EBNA-3A epitope are under way in Australia.

Recent Accomplishments and Developments

An animal model for acute and persistent Epstein-Barr virus infection has been developed. The principal primate model for studying EBV infection and evaluating vaccines—the cottontop tamarin—has a number of shortcomings, not the least of which is its status as an endangered species. Frederick Wang and coworkers at Harvard Medical School have developed a new model that may be useful for studying vaccines, as well as the pathogenesis and treatment of EBV infection and associated oncogenesis. A rhesus herpesvirus that is similar to EBV and that is naturally endemic in rhesus monkeys was used to orally infect naive animals from a pathogen-free colony. This animal model reproduced key aspects of human EBV infection, including oral transmission, atypical lymphocytosis, lymphadenopathy, activation of CD23(+) peripheral blood B cells, sustained serologic responses to lytic and latent EBV antigens, latent infection in the peripheral blood, and virus persistence in oropharyngeal secretions.

Next Steps/Challenges Ahead/What's on the Horizon

It is not known whether vaccination with gp220/350 alone will be adequate to protect against primary infection, and whether such a protective response would be effective against EBV-associated tumors where the expression of viral gene products is both limited and different. Little has been reported on the use of antigens other than gp220/350 in candidate subunit or recombinant vaccines. Further work is also needed on defining the CTL specificities that a candidate vaccine should target. A new phase I trial of a gp220/350 subunit from SmithKline Beecham Biologicals is expected to commence shortly. Results from the Australian phase I evaluation of peptide vaccination are pending.

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Parasitic, Tropical, Vector-Borne, and Zoonotic Infections

Overview

Parasitic diseases continue to plague literally billions of people in the modern world, killing millions annually and inflicting debilitating injuries such as blindness and disfiguration on additional millions. The World Health Organization (WHO) estimates that 1 person in every 10 is infected with a major tropical disease, and approximately 1 person in 4 harbors parasitic worms. These infections exact an enormous toll on world health and the global economy, particularly in less developed countries, where the diseases are often cited as a major impediment to economic progress. Despite efforts at control, some parasitic diseases are actually becoming more widespread owing to development of drug resistance and to changing water and land management policies that have brought humans in closer contact with parasite vectors.

In addition, parasites remain a public health concern in the United States and other developed countries. Many human parasites are widely distributed in this country, but infections remain subclinical because of other influences such as good nutrition and hygiene practices. In immunologically immature or immunosuppressed populations, however, parasitic infections represent a significant cause of morbidity and mortality. Moreover, symptomatic parasitic infections are becoming more widely observed in the United States as a consequence of the increased numbers of Americans traveling abroad and immigrants from endemic areas. Recently, isolated endemic foci of some exotic parasitic infections (e.g., malaria and leishmaniasis) have been reported in the United States.

Malaria

Malaria is a major health problem in the world's tropical areas, where it is responsible for high rates of morbidity and mortality, especially in children and pregnant women. The yearly incidence of malaria is estimated at approximately 300 to 500 million cases, resulting in 1.5 to 3 million deaths annually. The development of a vaccine has been given high priority, because the control of malaria is difficult and has been further inhibited by the selection of drug-resistant parasites and insecticide-resistant mosquito vectors. Much work is now being done to determine the immunologic response to infection and to elucidate the protective epitopes that can be used in the construction of a synthetic or recombinant malaria vaccine. Such vaccines target the infective sporozoite stage, the replicating liver or blood stages, or the sexual stages that are infective for the mosquito vector.

Over the past few years, an increasing number of malaria vaccines have been tested in limited human trials. Trials done in the 1970s with irradiated sporozoites provided very good protection in volunteers challenged with infectious parasites. Similar studies were subsequently conducted to take advantage of improved immunological techniques for the identification of immune correlates of resistance. Four of five vaccinated volunteers were protected, as measured by the absence or delayed onset of parasitemia following challenge infection. Protected individuals developed antibodies to sporozoites, including the repeat region of the circumsporozoite (CS) protein, and to antigens expressed by liver-stage parasites. T cell proliferation, cytotoxicity, and cytokine production also have been observed in response to recombinant CS protein.

In studies in animal models, CS-based synthetic peptide and recombinant vaccines conferred protection when given with strong adjuvants. Early trials with CS-based synthetic peptide and recombinant vaccines demonstrated enough immunogenicity to warrant challenge studies; when such studies were carried out, however, the degree of protection was disappointing. These results were interpreted to mean that better immunogenicity could be developed if more powerful adjuvants were available for use in humans.

During the 1990s many studies were carried out with various candidate malaria vaccine formulations comprising different adjuvants. These studies either failed to demonstrate adequate immunogenicity to warrant further evaluation or failed to demonstrate adequate protection against challenge infection. In early 1997, however, investigators working at the Walter Reed Army Institute of Research reported that a novel combination vaccine based on recombinant fusion proteins of the CS protein and the hepatitis B surface antigen could provide protection against challenge infection when the vaccine was formulated with an appropriate novel adjuvant. These results are encouraging in themselves and validate the importance of vaccine formulations incorporating strong adjuvants that elicit appropriate immune responses. Nevertheless, much work remains to be done to extend these results to the development of an effective field vaccine.

An alternative approach that also appears promising is to identify specific regions of the CS protein that stimulate immune responses, and then to incorporate several copies of those regions into a synthetic structure called a multiple antigenic peptide (MAP). Several research groups are currently examining different MAPs for their potential as candidate vaccines. MAPs based on CS protein structures have been shown to elicit high antibody titers in animal models and are capable of boosting preexisting malaria-specific immune responses. A phase I clinical trial of a CS-based MAP vaccine developed initially at New York University is now in progress at the University of Maryland, and results are expected in early 1998.

One potential problem associated with evaluation of synthetic peptide-based vaccines such as MAPs is that genetic factors may limit immune responses to the vaccine. This is particularly important since, if the responsive individuals are not adequately represented in the initial immunogenicity study, the candidate vaccine might be rejected as nonimmunogenic. To address this issue in the above-mentioned phase I MAP trial, volunteers were prescreened for presumed immune response genes to ensure that an adequate number of responder individuals were included.

Recently, attention has been directed to the nonrepeat domains of the CS polypeptide. A genetically conserved region within these domains has been implicated in parasite attachment to liver cells. A genetically engineered CS-derived polypeptide in which the repeat region has been excised has been incorporated into liposomes and is undergoing clinical evaluation.

Although malaria vaccine efforts in the past have focused mainly on the humoral aspects of immunity, increasing attention is being directed to the important role played by T cells. In addition to enhancing antibody responses and conferring immunological memory, T cells also mediate cytotoxic immunity and induce the production of cytokines such as gamma interferon. CS-responsive T cell clones have been established from cells of vaccinees immunized with attenuated parasites; they may prove to be useful in future studies on the development of immune responsiveness. Epitopes of CS polypeptides recognized by helper T cells, as well as by cytotoxic T lymphocytes (CTL), have been identified and are being incorporated into recombinant vaccine candidates for further testing. To identify new candidate vaccine components, investigators recently have employed a new approach, called reverse immunogenetics. Using this technique, they identified a peptide component of a liver-stage parasite protein that is efficiently recognized by cytotoxic T cells from individuals who are resistant to severe malaria.

Several blood-stage vaccine candidates are now in preclinical evaluation. In studies in *Aotus* monkeys, candidates based on the 42 kilodalton and 19 kilodalton C-terminal fragments of the major merozoite surface protein (MSP-1) have elicited protection. A phase I clinical trial of a candidate based on the MSP-1 19 kD fragment has recently been carried out at the Baylor College of Medicine, and the results should be available in 1998.

Almost 10 years ago, a blood-stage vaccine developed in Colombia was reported to delay or suppress the onset of disease during trials in Colombia. In a randomized, double-blind trial conducted in Colombia, the vaccine was reported to have an overall efficacy of 40 percent. Two other clinical trials in South America have reported similar results. These studies, however, were carried out in areas of low or seasonal malaria transmission, and thus the utility of this vaccine in areas of high transmission and in other geographic locations has been questioned. To address these issues, randomized, double-blind, controlled clinical trials have recently been completed in Tanzania, The Gambia, and Thailand. In the

Tanzanian study the estimated efficacy of SPf66 was 30 percent but with wide variability. In both the Gambian and Thai studies, however, no significant efficacy was demonstrated. It is noteworthy, however, that since all of these trials addressed only selected clinical features of malaria (i.e., fever and high numbers of parasites in the bloodstream), the potential of SPf66 to reduce either malaria-attributable morbidity or mortality cannot be extrapolated from them.

Antigens of the sexual stages of the malaria parasite that may immunize for transmission-blocking activity also have been identified. One of them, a 25 kD molecule, when expressed as a recombinant construct in yeast, has shown efficacy in animal models. From these studies, however, it is clear that attaining and maintaining a high titer of transmission-blocking antibody are very likely to be important for efficacy. Experiments are currently under way to identify novel formulations that will address this issue. Phase I clinical testing of this vaccine candidate formulated with alum has been carried out, and results should be available in the near future.

It is obvious that, before the structure of an ideal vaccine can be developed, more details are needed on the immune response to malaria and the factors involved in protection, including the use of immunogenicity-enhancing adjuvants and carrier proteins. NIAID has encouraged research in this area with recent program announcements and other initiatives, and studies to expand existing knowledge in these areas are under way in a number of laboratories. In addition, novel vectors (e.g., modified viral vectors), delivery systems, and alternative strategies to prime and boost protective immune responses differentially are being investigated.

One exciting approach that is being developed for malaria as well as a number of other infectious diseases is a DNA-based vaccine. Such vaccines have the advantage that they may elicit both humoral and cellular arms of the immune response and that they may simplify evaluation of vaccines involving multiple different antigens. Thus, they may find utility at several stages in the vaccine identification and development process. Because DNA vaccines are so new, however, experience with them is limited, and the issues of safety, immunogenicity, and efficacy, especially in the long term, still need to be addressed. A phase I trial of a CS-based DNA vaccine is currently under way at the Naval Medical Research Institute.

Finally, as pointed out in a recent report from the Institute of Medicine, "Vaccines Against Malaria: Hope in a Gathering Storm," close coordination and collaboration of malaria vaccine development efforts could accelerate the process. Significantly, a number of efforts are under way to enhance and expand collaborative activities in malaria vaccine research and development. For several years now, NIAID staff members, along with representatives from the Centers for Disease Control and Prevention, the Department of Defense, and the U.S. Agency for International Development, regularly participate in the Federal Malaria Vaccine Coordinating Committee, an interagency working group that provides for timely exchange of information and collaborative efforts to accelerate malaria vaccine research and development. In an unprecedented meeting of scientists, administrators, and public health officials in January 1997 in Dakar, a number of priorities for malaria control, including malaria vaccine development, were identified. At subsequent meetings in The Hague, Netherlands, and London, U.K., representatives of interested public and private sector entities have begun to develop strategies for international collaboration in supporting malaria research and control in Africa. Finally, under the auspices of the Indo-U.S. Vaccine Action Program, U.S. and Indian scientists recently met in India to develop collaborations on malaria vaccine development.

Schistosomiasis

Schistosomiasis is another parasitic disease with a major human health impact. It is estimated that 200 million people worldwide are infected with this helminth, and approximately 600 million people live under conditions in which they are directly exposed to infection. Schistosomiasis is primarily a chronic disease associated with significant morbidity and loss of productivity; nevertheless, the mortality rate is estimated in the hundreds of thousands.

Much recent research on schistosomiasis has focused on the identification of candidate vaccine antigens. Several have now been shown to provide partial protection in a mouse model of infection with the human parasite *Schistosoma mansoni*, a form found in South America and Africa. Many antigens are molecules associated with the invasive larval stage of the parasite; such antigens were initially distinguished by their reactivity with protective monoclonal or polyclonal antibodies. They include the enzymes glutathione-S-transferase and triose phosphate isomerase (TPI), as well as a 38 kD antigen with prominent carbohydrate epitopes that are shared between the larval and egg stages.

Another promising candidate, calpain, was recently identified based on the ability of a T cell clone to transfer protection against challenge infection in mice. Several other antigens, whose identities have not yet been determined, also have demonstrated partial protective activity. Schistosome paramyosin, a muscle protein, has been shown to induce a protective cell-mediated immune response, based on the production of gamma interferon-activated macrophage effector cells. Several vaccine candidates are being tested for efficacy against *S. mansoni* in baboons. One, a 28 kD glutathione-S-transferase of *S. mansoni*, has been shown to reduce worm burden and/or egg excretion in baboons and cattle. A myosin-like antigen also has shown efficacy against *S. mansoni* in both mice and baboons; multiple antigen peptides (MAPs; see above), based on selected regions of TPI and a 23 kD antigen, also have shown promise as candidate vaccines against *S. mansoni* in mice.

Additional investigations on mechanisms to enhance the level of protective immunity achieved with purified native or recombinant-derived antigens are under way; such studies include examining the benefit of combining antigens or of varying the method used to present antigen to cells of the immune system. DNA-based vaccines are also being exploited to identify promising routes of administration, combinations of vaccines, and protective immune effector mechanisms. Recent studies carried out in Egypt, Brazil, and Kenya have identified antigen-specific immunologic correlates of resistance to reinfection in populations at risk. Based on these results, plans are now being made for further development of candidate vaccines, including pilot lot production according to good manufacturing practice guidelines and phase I clinical evaluation.

Other Parasitic Diseases

Candidate vaccine antigens have been identified for other parasitic diseases, including leishmaniasis, toxoplasmosis, amoebiasis, and filariasis. Leishmaniasis is caused by several species of protozoan parasites found in most areas of the world, but particularly in the tropics. In its severest forms, this disease can cause serious disfigurement as well as death, and the WHO estimates worldwide prevalence to be approximately 12 million cases. Several WHO-supported efficacy trials of vaccines based on a combination of whole, killed leishmania parasites and BCG are nearing completion. An alternative approach to developing attenuated leishmania vaccines based on gene replacement in *Leishmania major* is under investigation.

Two *Leishmania* surface antigens have been identified; they apparently serve as ligands for the attachment of the parasite to host macrophages, thereby enabling infection to be initiated. They consist of gp63, a glycoprotein with protease activity, and a glycoconjugate known as lipophosphoglycan. Both have been shown to induce protection in a mouse model of leishmaniasis. In addition, a 46 kD promastigote antigen, derived from *Leishmania amazonensis*, has been shown to protect mice when administered as the native molecule admixed with adjuvant or as a recombinant vaccinia construct.

Over the past several years NIAID-supported investigators have demonstrated that T lymphocyte-dependent host responses to the leishmania parasites determine whether the disease is progressive or self-limited in experimental animal models. More specifically, when a Th1 lymphocyte response (characterized by the production of cytokines, such as interleukin [IL]-2 or interferon-gamma) is dominant, the disease is self-limited, whereas when a Th2 lymphocyte response (characterized by the production of other cytokines, such as IL-4 and IL-5) is dominant, the disease is progressive. NIAID-supported investigators have demonstrated that incorporation of the cytokine IL-12, a specific stimulator of Th1 responses, into an experimental vaccine against leishmaniasis resulted in complete protection of susceptible mice against progressive disease. Neither IL-12 alone nor the experimental vaccine without IL-12 conferred protection. Thus, IL-12 may be useful as an adjuvant in vaccines designed to enhance Th1-dependent protective immune responses.

Recently, expression cloning has been used to identify a novel parasite antigen (LACK) that appears related to a family of enzyme receptors. When administered with IL-12, this antigen has also been shown to confer protection against leishmaniasis in susceptible mice.

DNA immunization is also being used to identify and validate candidate vaccine antigens for leishmaniasis. In mice protection against *L. major* has been demonstrated following immunization with DNA constructs encoding gp63 and LACK antigens.

Toxoplasmosis is primarily a disease of the central nervous system that affects individuals with immature or compromised immune systems. It usually is associated with neurological problems in the developing fetus; more recently, however, it has been identified as a major opportunistic infection in AIDS patients. The possibility of effective vaccination against this protozoan parasite was suggested by experiments showing that mice immunized with a temperature-sensitive mutant of *Toxoplasma gondii* were resistant to further infection with a potentially lethal strain. In addition, a major surface antigen of *T. gondii*, called p30, now has been cloned. This antigen has been shown to stimulate CTLs with parasiticidal activity *in vitro*. Purified native p30 recently has been demonstrated to protect mice against parasite challenge *in vivo*.

Amoebiasis, caused by invasion of the intestinal wall and gut-associated organs by the protozoan parasite *Entamoeba histolytica*, has been estimated to result in more than 100,000 deaths per year; the prevalence of infection may be as high as 50 percent in some developing countries. Recent studies have identified a galactose-inhibitable amoebic lectin involved in adherence of the parasite to the colonic mucosa. Gerbils immunized with this lectin showed a significant reduction in development of liver abscesses following infection, suggesting that this molecule might form the basis of a potential vaccine against amoebiasis. Investigators are working to identify the regions of the lectin that elicit protective immunity and to develop genetically engineered and recombinant subunit vaccines based on these regions. In addition, investigators are working to identify new antigens and delivery systems, especially those that would target mucosal immunity.

Lymphatic filariasis is endemic in many tropical and subtropical countries, where it is estimated to afflict approximately 90 million people. In its chronic form, this infection causes inflammation and blockage of the lymphatic system, resulting in the condition known as elephantiasis. Immunization with several *Brugia malayi* antigens has been demonstrated to facilitate the clearance of bloodstream forms (microfilariae) of the parasite in animal models. One such antigen is paramyosin, a 60 kD antigen. In addition, filarial collagen has been shown to partially inhibit the development of infective larvae into adult worms.

Dengue

Dengue (DEN) viruses are the most widespread arthropod-borne viruses (arboviruses). They are members of the Flaviviridae family, which includes more than 70 related—but distinct—viruses, most of which are mosquito-borne. Other major pathogens in this family include yellow fever (YF) and Japanese encephalitis (JE) viruses. In 1997, DEN was present on most continents, and over one-half of all United Nations member-states were threatened by DEN. Epidemics continue to emerge, and this virus causes severe infections in areas where periodic epidemics did not occur previously. The disease will continue to spread as newly urbanized areas become infested with mosquito vectors. In those areas where DEN is endemic, more than 1.5 billion people—including about 600 million children—are at risk. Each year, it is estimated that from 35 million to 60 million people are infected with DEN and that 2,000 to 5,000 children die from this viral infection. These figures most likely underestimate the scope of this problem.

There are four closely related, but serologically distinct, DEN viruses (types 1 through 4). Since there is no cross-protection between the four types, a population could experience a DEN-1 epidemic in one year, followed by a DEN-2 epidemic during the next year. Primary infection with any serotype often causes a debilitating—but usually nonfatal—form of illness. To date, antiviral drug chemotherapy has not been successful; consequently, most currently used forms of therapy are supportive in nature.

Some infected patients experience a much more severe—and often fatal—form of the disease, called dengue hemorrhagic fever (DHF). Unlike other infectious diseases, the presence of antibod-

ies after recovery from one type of DEN infection is believed to predispose a percentage of individuals to contract the more severe form of disease (DHF) when infected by a second, different DEN virus serotype. Thus, in some people infected during a DEN epidemic, antibody-mediated "immune-enhancement" could cause some children to develop fatal DHF during a subsequent outbreak of the disease caused by another DEN type. Although all age groups are susceptible to DEN fever, DHF is most common in children, and usually occurs in about 1 to 10 percent of all hospitalized cases.

Dengue viruses are prevalent throughout the tropics, where the urban-dwelling mosquito *Aedes aegypti* is a major vector. Although the virus typically circulates in endemic cycles, it periodically causes acute, widespread epidemics, in which large percentages of the population may be infected. An example was the 1987 epidemic in Thailand, which officially recorded 174,285 cases of DEN; most involved children less than 15 years of age. Dengue caused 1,007 reported deaths in these children. That year, aside from diarrhea and fever, DEN was the third leading cause of illness in children (154,381 cases) and the leading cause of childhood death (925 cases). DHF has emerged as an ever important public health problem in Southeast Asia as new waves of epidemics occur; this appears to be happening in the Western Hemisphere as well. In the Americas, the first large grouping of cases of severe DHF occurred in 1981. They were associated with a DEN-2 epidemic in Cuba that followed the DEN-1 epidemic of 1977. During the 1981 outbreak in Cuba, 116,151 hospitalized cases of DEN fever were reported and 10,312 cases were classified as severe DHF; 158 deaths (many in adults) were reported. More recently, parts of the Caribbean and South and Central America have experienced major outbreaks of DEN, with cases of fatal DHF now commonly reported from many countries.

Dengue continues to spread or emerge into areas previously considered to be endemic but not usually associated with major outbreaks of the disease. The westward expansion of DEN in Asia was first documented in the late 1980s by the appearance of epidemics of DEN in India and Sri Lanka. Africa and the Middle East also were considered to be areas with a low incidence of DEN; however, DEN has emerged in these areas in the early 1990s, as evidenced by the widespread occurrence of DEN infections in U.S. military personnel stationed in Somalia, as well as by reports of DEN in Saudi Arabia.

The control of DEN will be possible only after an efficient vaccine has been developed, since attempts to eradicate mosquito vectors have not been successful in developing countries. Clearly, the phenomenon of immune-enhancement may be a major problem in developing an effective vaccine for DEN. It suggests that instead of a monotypic vaccine, one may have to prepare a multivalent vaccine against all four serotypes of the DEN virus to avoid inducing monotypic enhancing antibodies that might lead to DHF associated with subsequent natural infections caused by other DEN types. The effects of administering a live-attenuated vaccine to a population with preexisting enhancing antibodies are another potential problem that remains to be examined in a systematic manner.

NIAID is now funding several projects that address basic virological and immunological aspects of flavivirus infections in general, and DEN infections in particular. The World Health Organization (WHO) is also funding vaccine development programs, and DEN vaccine development programs are in place at a limited number of vaccine manufacturers and small biotechnology companies. The United States Army has had a productive, long-term research program aimed at developing a DEN vaccine. However, funds to support this program have been threatened in recent years.

Progress in research on DEN has been slowed, mainly because these viruses grow poorly in cell culture, and there is no acceptable animal model for DEN infection or DHF. NIAID funds several extramural and intramural projects studying basic virological and immunological aspects of flaviviruses such as YF, DEN, and JE virus. Discoveries from these projects cross-fertilize vaccine studies on YF, DEN, and JE, and the reader is referred to details reviewed in the sections on these particular vaccines. In brief, some of the most promising basic molecular studies that might be applied to the development of an improved vaccine revolve around the development of full-length, infectious YF, DEN, and JE cDNAs. Information from studies using this infectious clone has been combined with sequence data and immunological data to yield new insights into important antigenic regions on the DEN virion. The recent determination of the three-dimensional structure of the E protein of another flavivirus (tick-borne encephalitis virus) has allowed formulation of an even more sophisticated model for understanding antigenicity and pathogenicity of flaviviruses. Hopefully, this research could yield efficient and less costly ways to manufacture safe flavivirus vaccines.

Flavivirus vaccine development research has focused on five areas: live-attenuated or inactivated vaccines, infectious clone-derived vaccines, immunogens vectored by various recombinant systems, subunit immunogens, and nucleic acid vaccines.

The most promising set of live-attenuated DEN vaccines has been developed in Thailand with support of the WHO. Preliminary trials in adults and children in Thailand were encouraging, with the tetravalent vaccine inducing broadly cross-reacting antibody in 80 to 90 percent of the subjects. This vaccine has been transitioned to commercial development by agreements with Pasteur Merieux Connaught, and commercial lots have been manufactured, and phase I testing is under way in collaboration with Walter Reed Army Institute for Research (WRAIR).

Because of the success of flavivirus inactivated vaccines against JE in Japan, and tick-borne encephalitis in Australia, attempts have been made to develop a killed DEN vaccine. However, because of difficulties in growing high titers of DEN in cell culture, early attempts to make inactivated products were not successful. Recently, WRAIR scientists have utilized certified vero cells and serum-free media to grow DEN to high titers. A prototype DEN-2 inactivated vaccine purified and concentrated from these cells induces protective levels of antibodies in mice and monkeys. Further testing is planned.

Infectious clones of DEN, JE, and YF are being combined to produce chimeric vaccines, and preliminary mouse studies are encouraging. Scientists at the National Institutes of Health and in Australia have also attempted to alter the genetic structure of the DEN clone to produce live-attenuated vaccine candidates. Mouse and monkey trials have been encouraging, and a number of potential vaccine candidates soon will be tested in phase I trials.

The most advanced studies of flavivirus immunogens delivered by poxvirus vectors have been with JE virus to deliver antigenic JE proteins to humans in phase I trials. Further studies are needed, but these vectors induce both cellular and antibody immunity against JE. Preexisting immunity to the vector attenuated the response. To avoid this problem, vaccinia virus recombinants have also been used to generate subviral particles containing DEN and JE antigens. These particles elicit antibody in mice, but their potential as vaccines is still being explored.

Subunit vaccines for a variety of flaviviruses have been prepared in *E. coli*, baculovirus, yeast, and insect cell systems. The experience with DEN-containing *E. coli* products, and some other expressed products, was not promising. With *E. coli* DEN products, mice elicited good antibody titers, but monkey studies were not as successful. One lesson learned was that flavivirus proteins require extensive processing and folding during maturation. Studies to fine-tune various expression systems to yield more stable flavivirus immunogens are under way, and baculovirus expressed products and products from drosophila cells appear promising in early mouse testing.

Preliminary studies have been reported on a new nucleic acid vaccine for St. Louis encephalitis, a related flavivirus. PreM and E proteins have been expressed under control of the cytomegalovirus immediate early promoter. Mice immunized with this product developed disappointing levels of antibody but were protected against a live virus challenge. Research by the Centers for Disease Control and Prevention (Ft. Collins) and the U.S. Navy is attempting to further develop this approach for DEN. In the near future, this exciting area undoubtedly will be a focus of expanded vaccine research efforts.

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Japanese Encephalitis

Japanese encephalitis (JE) is endemic in parts of China, India, Korea, Nepal, Thailand, Vietnam, Kampuchea, Myanmar, the Philippines, Taiwan, Indonesia, Malaysia, Bangladesh, and Sri Lanka. Infection with this mosquito-borne virus is common; however, clinical disease occurs in only 1 of every 300 to 1,000 infections. These clinical cases have a case fatality rate ranging up to 40 percent, with severe neurological sequelae occurring in 10 to 30 percent of survivors. Like the closely related yellow fever (YF) and dengue (DEN) viruses, JE virus circulates in endemic cycles, which periodically erupt into major epidemics. Consequently, the incidence of infections caused by JE virus varies substantially and ranges from 10,000 to more than 50,000 cases per year. Estimates of about 1,000 cases per year have been reported in India, Nepal, and Sri Lanka. An annual morbidity of 6 to 10 cases per 100,000 inhabitants has been reported in heavily endemic areas such as Vietnam and Thailand.

Travelers, military personnel, and others temporarily assigned to endemic areas often require immunization. Exposure to JE virus has increased greatly with rapid economic development of the Pacific Rim countries and the large number of U.S. citizens visiting that region. The treatment of JE is mainly supportive, since antiviral drug chemotherapy has not been fully researched. In developed countries, the control of mosquito vectors or immunization of host reservoirs has limited the spread of virus, but these public health measures have been difficult to accomplish in developing countries.

An inactivated virus vaccine exists and has been used successfully to reduce the incidence of JE in Japan, Taiwan, and Korea. Currently mass-produced and licensed in Japan, the vaccine has been tested under various experimental protocols. The vaccine is made by Biken, was licensed in the United States in late 1992, and is also distributed by Connaught Laboratories. It consists of partially purified, formalin-inactivated JE virus that is propagated in mouse brain tissue. It requires a series of three to five injections to stimulate immunity. A new, live-attenuated vaccine (SA 14-14-2) has been developed and tested in China. It appears to be safe and efficacious in annual Chinese immunization programs involving millions of children. For international approval, efforts are under way to reconfirm safety and efficacy in carefully monitored trials in infants and children from 1 to 6 years of age. A recent review of 13,000 vaccinated and control children in Chengdu Province in China indicated low rates of acute systemic and local side effects, and no central nervous system infections were reported. The vaccine is produced in primary hamster kidney cells. Production issues remain a question since this is not a widely accepted substrate for the production and licensure of vaccines in some countries, and the vaccine is not produced under good manufacturing practice conditions. Further research is also needed to examine the vaccine's thermostability, its ability to revert to a more virulent form of the virus, its efficacy in children with maternal antibody, and its immunogenicity when used in combination with other vaccines.

NIAID currently funds several extramural and intramural projects studying basic virological and immunological aspects of flaviviruses such as YF, DEN, and JE virus. Discoveries from these projects cross-fertilize vaccine studies on YF, DEN, and JE, and the reader is referred to details reviewed in the sections on these particular vaccines. In brief, some of the most promising basic molecular studies that might be applied to the development of an improved vaccine revolve around the development of full-length, infectious YF, DEN, and JE cDNAs. Information from studies using this infectious clone has been combined with sequence data and immunological data to yield new insights into important antigenic regions on the JE virion. The recent determination of the three-dimensional structure of the E protein of another flavivirus (tick-borne encephalitis virus) has allowed formulation of an even more sophisticated model for understanding antigenicity and pathogenicity of flaviviruses. Hopefully, this research could yield efficient and less costly ways to manufacture safe flavivirus vaccines.

Vaccine development research has focused on five areas: live-attenuated or inactivated vaccines, infectious clone-derived vaccines, immunogens vectored by various recombinant systems, subunit immunogens, and nucleic acid vaccines.

As mentioned above, further safety and efficacy studies are planned for SA 14-14-2. Additionally, SA 14-14-2 itself is being molecularly modified using infectious clones to produce a vaccine that is highly stable to reversion. Infectious clones of JE and YF are being combined to produce chimeric vaccines, and preliminary mouse studies are encouraging.

Poxvirus vectors have been employed to deliver antigenic JE proteins to humans in phase I trials. Further studies are needed, but these vectors induce both cellular and antibody immunity against JE. Vaccinia virus recombinants have also been used to generate subviral particles containing JE antigens. These particles elicit antibody in mice, but their potential as vaccines is still being explored.

Subunit vaccines for a variety of flaviviruses have been prepared in *E. coli*, baculovirus, yeast, and insect cell systems. The experience with DEN containing *E. coli* products and some other expressed products was not promising. With *E. coli* DEN products, mice elicited good antibody titers, but monkey studies were not as successful. One lesson learned was that flavivirus proteins require extensive processing and folding during maturation. Studies to fine-tune various expression systems to yield more stable flavivirus immunogens are under way, and baculovirus expressed products and products from drosophila cells appear promising in early mouse testing.

Preliminary studies have been reported on a new nucleic acid vaccine for St. Louis encephalitis, a related flavivirus. PreM and E proteins have been expressed under control of the cytomegalovirus immediate early promoter. Mice immunized with this product developed disappointing levels of antibody but were protected against a live virus challenge. In the near future, this exciting area undoubtedly will be a focus of expanded vaccine research efforts.

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Leprosy

Leprosy, a chronic infectious disease caused by *Mycobacterium leprae*, has been a scourge of mankind since ancient times. It mainly affects the skin, peripheral nerves, and mucosa of the upper respiratory tract and eyes, often causing dramatic disfigurement and disability if untreated.

Mycobacterium leprae is an acid-fast, rod-shaped bacillus related to the bacterium that causes tuberculosis (*Mycobacterium tuberculosis*). Research on this bacterium has been markedly hampered by a continuing inability to culture it *in vitro* and its extremely slow doubling time (the slowest known for any prokaryote)—approximately 13 days. The bacilli can be propagated in the foot pads of nude mice, but the preferred animal model of disseminated disease is the nine-banded armadillo, which poses significant technical challenges of its own.

In the United States there are an estimated 6,500 cases of leprosy, including both those undergoing treatment and those off treatment, 640 registered cases and 157 new cases reported in 1996. The World Health Organization (WHO) estimates that in 1996 there were 1.2 million cases worldwide, with approximately 570,000 new cases detected. India, Indonesia, and Myanmar account for 70 percent of the world's cases of leprosy. Other "hot spots" for this disease continue to exist in Africa (the second most affected region), Brazil and Colombia in Latin America, and parts of central and eastern Europe. Leprosy is still considered endemic in 55 countries.

Dapsone was discovered to be effective against leprosy in the 1940s, but dapsone-resistant *M. leprae* gradually emerged, stimulating the recent development of multidrug therapy (MDT) for leprosy. Leprosy patients are classified on the basis of clinical manifestations and skin smear results into paucibacillary (PB) and multibacillary (MB) cases. Standard MDT consists of rifampicin, clofazimine, and dapsone, given in a 6-month regimen for PB disease and a 2-year regimen for MB leprosy. A United Nations Development Programme/World Bank/WHO multicenter trial recently demonstrated that PB patients with a single skin lesion can be cured with a single dose of rifampicin, ofloxacin, and minocycline. WHO has also indicated that it may be possible to adequately treat MB disease with a 12-month rather than a 24-month course of standard MDT. The new regimens represent significant practical advances in the effort to control leprosy.

WHO has declared the goal of eliminating leprosy worldwide by the year 2000, defined as reducing case rates to less than 1 per 10,000 population. Although MDT is bringing this ideal closer to reality than had been thought likely even a few years ago, many investigators believe true elimination or eradication of leprosy will not be possible without vaccination.

Major priorities in leprosy research are to develop improved diagnostics, especially a sensitive and specific skin test; to further the understanding of the basic pathogenesis and epidemiology of the disease (it is not even clear how the disease is transmitted, or whether there is a significant nonhuman reservoir); and to develop alternate treatments and an effective vaccine.

Currently there are only a handful of candidates in the leprosy vaccine development pipeline. One of these is the antituberculosis vaccine, Bacille Calmette-Guérin (BCG). The Karonga Prevention Trial Group recently published the results of a double-blind, randomized, controlled trial of single BCG, repeat BCG, or combined BCG and killed *M. leprae* vaccine in the prevention of leprosy and tuberculosis in Malawi. BCG vaccination is standard practice in many countries for prevention of tuberculosis, although its effica-

cy appears to be highly variable. The factors responsible for this variability are not clear; differences in BCG strain, environmental factors, and host genetics have all been proposed. The effectiveness of BCG in preventing leprosy has also been demonstrated in some settings but remains controversial. A previous study by the Karonga Prevention Trial Group in the same part of Malawi demonstrated that a single BCG vaccination afforded approximately 50 percent protection against leprosy but none against tuberculosis. The present study demonstrated that a second dose of BCG afforded an additional 50 percent protection against leprosy compared to a single BCG vaccination. The addition of killed *M. leprae* did not improve the protection afforded by a primary BCG vaccination. There was no evidence that any of the trial vaccines afforded protection against pulmonary tuberculosis. These data demonstrate that BCG can confer significant protection against leprosy, even in a setting where it does not protect against pulmonary tuberculosis.

A second approach being pursued in antileprosy vaccine development is the identification of major protective antigens and the use of these as the basis of subunit or recombinant BCG or vaccinia virus vector vaccines. As a recent example of such studies, one such protein, the 35 kilodalton (kD) protein of *M. leprae,* was recently identified as a major target of the human immune response to this pathogen. The 35 kD protein was expressed in the relatively fast-growing *M. smegmatis* and shown to resemble the native antigen in forming multimeric complexes and in being recognized by monoclonal antibodies and sera from leprosy patients. The *M. smegmatis*-derived recombinant antigen was recognized by almost all leprosy patients or contacts studied, via a T cell proliferative or immunoglobulin G antibody response, but not by most tuberculosis patients. These findings suggest the *M. leprae* 35 kD protein is a major and relatively specific target of the human immune response to *M. leprae* and holds promise as a component of a potential antileprosy subunit, recombinant, or DNA vaccine.

Another approach to leprosy vaccine development being investigated is the use of live atypical mycobacteria, such as *Mycobacterium w* or *M. habana*, to elicit a cross-protective immune response, and the testing of recombinant BCGs expressing other *M. leprae* antigen(s). Clinical testing of all these candidates would be vastly improved by identification of correlates of human protective immunity. Sequencing of the *M. leprae* genome is under way and should provide a significant boost to leprosy research, in general, and vaccine development, in particular—even more so than for many other microbial pathogens because of the extraordinary challenges involved in investigating this noncultivatable bacterium.

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Lyme Disease

Background

Lyme disease (borreliosis), which is caused by the spirochete *Borrelia burgdorferi*, was first recognized as an infectious disease in 1975. It is the most common tick-borne infection in the United States. Provisional data indicate that a total of 16,461 cases were reported to the Centers for Disease Control and Prevention (CDC) during 1996. In comparison to the 11,700 cases reported in 1995, this represents an overall increase of 41 percent. Although some of this increase may be attributed to changes in surveillance or disease reporting, it may also represent a true increase in the incidence of infection that may be explained, in part, by climatic changes (e.g., increased rainfall) that influence tick populations and increased exposure to infected ticks.

Current Status of Research and Development

NIAID has supported an extramural research program on Lyme disease since 1985. The program has grown from 2 research grants in 1985 to more than 45 grants and contracts in 1997. The objectives of the Lyme disease program that pertain to vaccine research and development include:

- Characterization of host immune responses expressed both during infection and after deliberate immunization with relevant microbial antigens to understand more fully the basis for the development of protective immunity, and
- Identification and characterization of virulence factors, as well as major cell surface components for possible use as vaccine candidates.

Recent Accomplishments

Two vaccines, consisting of a recombinant outer surface protein (OspA) of *B. burgdorferi*, were recently tested independently in multicenter, double-blind, placebo-controlled, phase III clinical trials. Although the clinical trials' design of these candidates differed, each of the studies involved more than 10,000 subjects (ages 15 to 92 years) from regions of the United States where Lyme disease is endemic. In one study, volunteers were given three injections (at 0, 1, and 12 months) of an adjuvanted lipidated-OspA (L-OspA) vaccine (or placebo) prepared by SmithKline Beecham (SKB) Pharmaceuticals and Biologicals. Preliminary results demonstrated a vaccine efficacy of 79 percent for those given three injections of vaccine, whereas partial protection, with a vaccine efficacy of 50 percent, was noted for those who received two doses of vaccine. Vaccination was associated with local or general reactions, most of which were mild in severity and self-limited. In the second study, 10,306 volunteers were given two doses (1 month apart) of an OspA vaccine (or placebo) prepared by Pasteur Merieux Connaught and Co., in the spring of 1994. The following year, 7,518 of the volunteers received a third dose of vaccine or placebo (0-, 1-, and 12-month schedule). All volunteers were observed for both clinical and laboratory evidence of infection for two Lyme disease seasons. Vaccine efficacy for adults less than 59 years of age after two or three doses of vaccine was 82 percent and 100 percent, respectively. The vaccine was generally well tolerated with an acceptable safety profile. Efficacy was not demonstra-

ble in volunteers over 60 years of age after two doses of vaccine, although an efficacy rate of 75 percent was observed for those given three doses of vaccine. In view of the promising results obtained, both manufacturers announced that their vaccines have been offered to volunteers who had been given placebo in these studies and both companies have applied to the U.S. Food and Drug Administration for licensure of their vaccines.

It should be noted that since OspA is not expressed by *B. burgdorferi* in the mammalian host, preexisting antibody to OspA in immunized individuals acts mainly by binding to spirochetes in the midgut of the infected tick and prevents the migration of *B. burgdorferi* to tick salivary glands. In this way the vaccine works to prevent transmission of infection to the mammalian host with the tick's subsequent feedings. Although antibody to OspA has been demonstrated to provide a first line of defense by blocking transmission, many believe that the addition of other borrelial immunogens, especially those that play a major role in virulence and/or the pathogenesis of this infection, may be needed to increase the efficacy of a vaccine against Lyme borreliosis. Several preclinical studies are in progress to identify and characterize such virulence antigens and evaluate their potential use in candidate vaccines.

The intradermal injection of rabbits with viable *B. burgdorferi* regularly results in the development of a characteristic skin reaction (erythema migrans or EM), dermal infection, and visceral dissemination of spirochete. Within 5 months, EM as well as all signs of dermal and visceral infection are eliminated, and rabbits become immune to subsequent reinfection. In view of these findings, a detailed study was conducted to compare such infection-derived immunity with that obtained after immunization with the lipidated recombinant OspA vaccine now being evaluated in humans. In these studies, 4 of 11 OspA-vaccinated rabbits, challenged intradermally at each of 10 sites with 10^5 low-passage viable cells of *B. burgdorferi*, developed EM, as well as dermal and disseminated infection. After identical challenge, 2 of the 11 infection-immune rabbits developed dermal infection, but not EM or disseminated infection. Antibody titers against OspA did not correlate with immune status for either OspA-vaccinated or infection-immune rabbits. The levels of antibodies against OspA were relatively low in the infection-immune group before challenge. These findings suggest that vaccination with OspA may result in a state of "partial immunity" that could mask the development of early signs of infection EM, possibly enabling the disease to progress to a more disseminated, chronic form of infection—a theoretical possibility that merits further study. These findings also imply that borrelial antigens other than OspA (e.g., virulence antigens) may in fact play a key role in generating protective immunity.

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Rabies

Rabies, an ancient human pathogen, continues to be a significant international health problem. In the United States, the emergence of rabies continues, especially in the raccoon population along the east coast. About a dozen human fatalities occur annually in the United States, and the number of post-exposure treatments is rapidly increasing, with a substantial associated financial burden. Globally, attention recently has been drawn to potentially significant underreporting of rabies in developing countries. In these areas, a number of vaccines, which vary in quality, are produced nationally and regionally, but post-exposure treatment remains costly and often beyond the financial reach of those exposed. The need remains for economic, safe, and effective vaccines suitable for mass immunization of domestic animals and wildlife.

Limited studies aimed to improve vaccines are under way with NIAID support. Vaccinia recombinants continue to be studied as an oral vaccine for wild animals, and attempts are in process to develop a nucleic acid vaccine for possible oral administration. To date, large field trials of vaccinia recombinant wildlife vaccine have shown promise, but further studies are needed to better establish efficacy and carefully define safe, optimal application of the vaccine. Research on post-exposure prophylaxis focuses on developing a one-shot, easily administered vaccine and on safe, inexpensive, carefully defined rabies virus-specific immunoglobulins. Although not now available, an antiviral drug against rabies might be useful for post-exposure prophylaxis.

Interestingly, in the United States, many recent human victims did not report a bite by a potentially rabid animal. Often the persons reported just "chasing bats" from their house. In many of these cases, the virus isolated was related to the strain found in silver haired bats. It is thought this bat could be a significant reservoir for U.S. rabies, and that its bite often might go unnoticed. However, some health officials have questioned whether exposure might have occurred by inhalation of bat excretions. Although the guidelines for commencing post-exposure treatment are well established for exposure to domestic animals, updated guidelines will have to be developed for exposure to wildlife, particularly bats.

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Yellow Fever

Yellow fever (YF) was first distinguished from other tropical febrile diseases during the 1647–49 epidemics in the Americas. Since then, it has raged as periodic epidemics in the Americas and Africa. The YF virus is mosquito-borne and in humans produces a clinical disease that starts with the sudden onset of acute fever, followed by a second phase of hepatorenal dysfunction and hemorrhage. Mortality rates vary widely, usually from 20 percent to 80 percent of all cases.

During the latter half of the 20th century, the disease circulated in an endemic, sylvatic cycle in the Americas, usually infecting up to 500 unvaccinated forest workers per year. By contrast, YF in Africa periodically explodes from its endemic cycle to infect large numbers during major epidemics. The highly successful mosquito eradication campaigns of the early 20th century effectively eliminated urban YF epidemics in South America and limited persistence of the virus to a monkey-mosquito cycle in jungle areas. However, the disease now appears to be slowly reemerging from the forest into those parts of South America where the vector, *Aedes aegypti*, has reinfested urban areas.

In the late 1980s, the worldwide total of cases of YF (with case fatality rates usually near 50 percent) represented the greatest number reported to the World Health Organization (WHO) during any 5-year period since 1948. Numerous studies showed that, in Africa, only a small number of cases of YF are reported. Ironically, 1988 marked the 50th anniversary of the development of the attenuated vaccine for YF—a safe and efficacious YF vaccine now has been available since 1937–38.

The 17D YF vaccine was one of the first viral vaccines to be developed. It is a live-attenuated vaccine that is produced in eggs. After one injection, the vaccine induces protective immunity in more than 98 percent of vaccinees for a period of at least 10 years. Indeed, protection may be lifelong since neutralizing antibodies have been detected as long as 40 years after immunization. The vaccine is one of the safest viral vaccines ever produced. From 1945 through 1989, only 17 cases (1 fatal in a 3-year-old) of encephalitis associated with YF immunization have been reported worldwide. Because all but three of these occurred in children immunized at 4 months of age or younger, a review by a panel of experts recommended that the YF vaccine not be given before 6 months of age.

Two vaccine-based control strategies have been attempted in Africa during the past 40 years. The first consists of routine immunization, whereas the second involves emergency control measures that are implemented after the start of an outbreak. A routine, mandatory YF immunization program was begun in the early 1940s in French West Africa; as a result, the recurring pattern of epidemics in West Africa has been interrupted. However, this strategy was abandoned in 1960, when a post-outbreak, fire-fighting type of emergency immunization and control strategy was adopted. Since then, there has been a series of epidemics of varying severity. In recent years, it appears that, with the help of the WHO Expanded Programme of Immunization (EPI), more and more African countries have at least partially incorporated the YF vaccine into their immunization programs. Most give both the YF vaccine and measles vaccine to children at 9 months of age, since the simultaneous administration of both vaccines has been shown to be acceptable. Recently, the Global Advisory Group for the WHO-EPI reviewed the situation with YF and recommended that all 31 nations in the YF endemic area incorporate the vaccine into their routine immunization programs.

In South America, YF control strategies have been primarily based on reducing mosquito vectors by altering their breeding environment. Extensive studies on the maintenance of YF virus have shown that the virus exists in two cycles: an urban cycle involving humans and *Aedes aegypti* mosquitoes, and a sylvatic or jungle YF cycle involving forest primates, principally monkeys, and forest canopy mosquitoes, with human infections tangential to the transmission cycle. In the Western Hemisphere, urban YF was solely transmitted by *A. aegypti* mosquitoes. In 1901, eradication efforts directed toward *A. aegypti* mosquitoes were launched under the direction of Dr. William Gorgas in Havana. These eradication efforts, with concomitant reduction of YF, were extended throughout Central and South America in the early 1900s. The chain of urban *A. aegypti*-transmitted YF was successfully broken by the eradication program. The eradication of the vector, and the concomitant reduction of urban YF cases in the Americas, historically represents one of the world's most successful public health campaigns against infectious diseases. Unfortunately, *A. aegypti* has now reinfested most of South and Central America and occupies habitats just adjacent to the areas where endemic YF transmission occurs. A major threat is that this species could transmit YF in an urban cycle. The Pan American Health Organization is monitoring the need for incorporation of YF immunization into the EPI programs in South America. Some authorities believe serious consideration should be given to expanding YF immunization in South American EPI programs in an attempt to prevent the reemergence of urban YF.

The EPI program provides an excellent, effective way to deliver YF vaccines to a wider population at a reduced cost; however, despite the fact that the current YF vaccine is an excellent public health tool, further studies are needed to expand its value in controlling YF. The amount of vaccine available has been a limitation at times in the past. The number of surviving infants in the 1990s, in the countries where YF is a potential risk, will be approximately 18 million. Although the vaccine is made in a number of devel-

oping countries, including Senegal and Nigeria, only about 6 million doses are produced yearly in Africa. Newer technology, combined with efficient technology transfer, might help solve the problem of availability. The development of a cell culture-produced vaccine might result in increased vaccine production. One recent study of vaccine thermostability showed that further work on stabilizing the YF vaccine is needed since only 5 of 12 manufactured lots meet the WHO criteria for vaccine thermostability. More research is needed on the safety of combining this vaccine with other vaccines in a multiple-dose regimen for immunization.

NIAID currently funds several extramural and intramural projects studying basic virological and immunological aspects of flaviviruses such as YF, dengue, and Japanese encephalitis (JE) virus. Discoveries from these projects cross-fertilize vaccine studies on YF, dengue, and JE, and the reader is referred to details reviewed in the sections on these particular vaccines. In brief, some of the most promising basic molecular studies that might be applied to the development of an improved vaccine revolve around the development of a full-length, infectious YF cDNA. Information from studies using this infectious clone have been combined with sequence data and immunological data to yield new insights into important antigenic regions on the YF virion. The recent determination of the three-dimensional structure of the E protein of another flavivirus (tick-borne encephalitis virus) has allowed formulation of an even more sophisticated model for understanding antigenicity and pathogenicity of flaviviruses. Hopefully, this research could yield efficient and less costly ways to manufacture safe flavivirus vaccines. At a minimum, a clone-derived vaccine seed virus might reduce YF vaccine production lot diversity, improve quality control, and reduce the need for vaccine safety testing in primates.

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Respiratory Infections

Overview

Infections of the upper and lower respiratory tract are the major cause of acute illnesses in the United States and are responsible for more than 4 million deaths each year worldwide. In the United States alone, there are nearly 2 million cases of pneumonia each year resulting in 40,000 to 70,000 deaths. Most of the deaths caused by respiratory infections occur in developing countries, in children less than 5 years of age. The elderly and immunocompromised are also at an increased risk for developing a fatal respiratory infection. The difficulty in identifying the causative agent, the rapid global emergence of antibiotic-resistant organisms, and the increased awareness of atypical pathogens causing respiratory infections have complicated the typical treatment regimen for lower respiratory tract infections. Adequate clinical management of infections relies primarily on the rapid and accurate identification of the causative agent to avoid the indiscriminate use of antibiotics that ultimately favors the development of resistance to antimicrobial agents. Treatment of infections caused by antibiotic-resistant pathogens requires the use of more expensive and/or toxic drugs and usually requires longer hospital stays. Estimates of the cost of antibiotic resistance in the United States annually range as high as $30 billion.

Upper respiratory tract infections (URI) include infections of the nose, pharynx (throat), or middle ear such as the common cold, strep throat, sinusitis, or otitis media. URIs are very common in children worldwide but seldom have serious or life-threatening complications, with the exception of rheumatic fever, a sequela to strep throat. The common cold accounts for approximately 20 percent of all acute illness in the United States, with associated direct costs estimated at over $500 million per year. Otitis media represents a significant health burden in the United States. This illness, which is caused by a variety of etiologic agents including nontypeable *Haemophilus influenzae*, *Streptococcus pneumoniae*, respiratory syncytial virus (RSV), and influenza virus, also results in significant costs, as well as substantial morbidity and long-term effects on speech and language development in children. Lower respiratory tract infections (LRI) include infections of the larynx, trachea, bronchi, bronchioles, or lung; are associated with more severe illnesses such as influenza, bronchitis, pertussis (whooping cough), pneumonia, and tuberculosis; and are more likely to require hospitalization.

Tuberculosis is the leading cause of death due to an infectious disease worldwide and is responsible for 26 percent of all avoidable deaths between the ages of 5 and 59. In 1996, the World Health Organization (WHO) reported that an estimated 8.9 million people developed tuberculosis in 1995, bringing the global total of sufferers to about 22 million.

In the United States, pneumonia and influenza are the sixth leading cause of death, being responsible for 3.6 percent of all deaths. In the elderly, influenza-related pneumonia remains the primary cause of infectious disease-related deaths, and the WHO has estimated that among 15 million children under 5 years of age who die each year (96 percent of whom die in the developing world), approximately 30 percent die from bacterial or viral pneumonia. Approximately one-half of pneumonia deaths are attributable to bacterial infections (primarily *Streptococcus pneumoniae* and *Haemophilus influenzae*), whereas the remainder are caused by viral (primarily respiratory syncytial virus) or mixed viral and bacterial infections. The exact etiologic diagnosis, however, is very difficult to establish and crucial to initiate adequate therapy.

Pneumonia is also the second most common type of nosocomial or "hospital-acquired" infection, after those of the urinary tract. Pneumonia accounts for approximately 15 percent of all nosoco-

mial infections and has been associated with mortality rates of 20 to 50 percent. Nosocomial pneumonia can prolong hospitals stays by 4 to 9 days, with an estimated cost of $1.2 billion a year for the United States. With its reported frequency and high fatality rate and cost, nosocomial pneumonia is a major infection control problem.

RSV is the most frequent cause of serious viral respiratory tract infections (pneumonia and bronchiolitis) in infants and children under 4 years of age. It is a common cause of winter outbreaks of acute respiratory disease and results in an estimated 90,000 hospitalizations and 4,500 deaths each year in the United States.

According to data obtained from a 1994 health survey in the United States, there were more than 208 million acute cases of respiratory infections, including 14 million cases of bronchitis, accounting for an estimated 707 million restricted activity days, 345 million bed days, and 129 million days of work lost among employed persons over age 18. Respiratory infections were responsible for nearly 12 million visits to hospital emergency rooms, 5.9 million visits to hospital outpatient departments, and 93.5 million doctors' office visits. The burden of respiratory infections is not only on the loss of lives but also the drain it puts on health resources.

Infections with respiratory pathogens such as *H. influenzae* type b (Hib), group B streptococcus (GBS), and *Neisseria meningitidis* can lead to other diseases, not usually considered to be respiratory in nature. For example, before the introduction of Hib vaccines, Hib invasive disease caused 10,000 to 15,000 cases of meningitis per year in the United States with high morbidity and mortality. Systemic GBS infection during the first 2 months of life affects approximately 3 live births per 1,000 and results in about 12,000 cases annually in the United States. A substantial number of these infants die or have permanent neurologic sequelae from the meningitis associated with this infection.

A major goal of the NIAID Respiratory Diseases Program is to stimulate and support research that may lead to more effective and accepted prophylactic and therapeutic approaches for controlling respiratory infections and their subsequent systemic infections. Areas of interest include the development and licensure of vaccines and therapeutic agents for respiratory pathogens; stimulating basic research on the pathogenesis, immunity, and structural biology of respiratory pathogens; developing better and more rapid diagnostic tools; and understanding the long-term health impact of acute respiratory infections in various populations.

Chlamydia pneumoniae

Background

Chlamydia pneumoniae (CP) is recognized as an important cause of acute respiratory tract infections including pharyngitis, sinusitis, and bronchitis; in addition, severe systemic infections, while uncommon, do occur. It is the third or fourth most common cause of pneumonia, accounting for approximately 10 percent of all cases of pneumonia and 5 percent of all cases of bronchitis in the United States. Infection is usually asymptomatic, especially in the younger age groups but can be seen also in the elderly. Most children become infected between the ages of 5 and 14 years. However, the disease is more severe and has the highest incidences in the elderly; case fatalities of 6 to 23 percent have been reported in these populations. Transmission of the disease is by person to person via respiratory droplets. Although CP has been isolated from the nasopharynx of healthy individuals, the rate of asymptomatic carriage in the normal population is unknown. Epidemics of pneumonia caused by CP have been documented for a number of geographic locations mostly in northern Europe. Studies indicate that approximately 40 to 60 percent of the adult population worldwide have antibodies to *C. pneumoniae,* suggesting that the infection is universal.

Clinical disease manifestations associated with CP extend beyond respiratory illnesses. For instance, there has been a recent association of CP with cardiovascular disease. Initially, this association was made on the basis of elevated IgG and IgA antibodies and increased chlamydial lipopolysaccharide (LPS)-containing immune complexes in 50 to 60 percent of patients with coronary heart disease or acute myocardial infarction as compared to 7 to 12 percent in control patients. Subsequent to these studies, several other investigators in the United States and other countries have reported similar findings in patients with coronary heart disease and come to similar conclusions. Also, CP has been associated with Guillain-Barré syndrome, endocarditis, and the exacerbation of asthma. Infections caused by CP can occasionally result in shock and multiorgan dysfunction syndrome and have been associated with acute pulmonary exacerbation in some patients with cystic fibrosis. CP has been isolated from immunosuppressed patients such as those with AIDS; however, its role as an opportunistic pathogen is unclear. Thus, infections attributed to or associated with CP represent a serious impact on the public health in the United States and worldwide. Although conventional antibiotic therapy has been shown to be effective against CP, recurrent infections have been shown to occur following treatment. Consequently, alternative strategies such as vaccine development should be considered. To date, there are no published controlled clinical trials of treatment for CP infection.

As a group, Chlamydia cause important infections in animals and humans. Chlamydia are distinguished from other bacteria by having a unique life cycle with an orderly alternation of dimorphic forms that are functionally and morphologically distinct. The infectious form, known as the elementary body (EB), is specialized for invasion into susceptible host cells. Following endocytosis, the EB differentiates into a larger form called the reticulate body (RB). Once inside the cells, the organism resides inside membrane-bound vesicles and can modify the inclusion membrane, resulting in evasion of lysosomal fusion and immune detection. Chlamydia grow only intracellularly and require and use substrate and energy pools of the host cells for growth and as such have been termed "energy parasites." A special property of Chlamydia is the ability to persist in cells and may, therefore, cause latent or chronic infections. The chronic state may be related to the ability of the organisms to develop into morphologically aberrant forms that do not divide or differentiate into EBs; this state may favor the development of immune-mediated diseases and the avoidance of host defense strategies. Studies show that these aberrant forms can be induced experimentally by the administration of cytokines such as interferon-gamma and are characterized by the absence of typical inclusions, low-grade infectivity, and altered expression of key membrane surface proteins. There is a lack of understanding

about the mechanisms by which Chlamydia cause disease, and very little information is available on factors associated with virulence. The organisms possess two major surface proteins, namely an outer membrane protein (OMP1) and LPS. Chlamydial LPS has a low endotoxic activity when compared to the LPS of Enterobacteria; however, the role of LPS or the OMPs in pathogenesis is not defined. Studies indicate that the aberrant form has an altered expression of the OMP. Chlamydia do not have a peptidoglycan layer but interestingly contain penicillin binding proteins on their cell walls and express a number of heat shock proteins (hsp). Future studies should be directed at determining whether any of these microbial components could serve as vaccine targets for CP infections.

Certain characteristics help distinguish *C. pneumoniae* from two other closely related organisms: *C. trachomatis* and *C. psittaci*. CP is separated from *C. trachomatis* and *C. psittaci* on the basis of DNA homology. Thus far, CP has been found to have one immunotype TWAR (derived from the first two strains, TW-183 and AR-39). However, more recent studies indicate that CP strains are antigenically different from each other, suggesting that more than one serovar of CP exist. The organism forms dense round inclusions in tissue culture cells that are more similar to *C. psittaci* than to *C. trachomatis*. Also, CP has a characteristic pear-shaped EB that is surrounded by a periplasmic space. Further, ultrastructural studies of the entry of CP organisms into HeLa cells show differences between CP and *C. trachomatis* and *C. psitta*ci in terms of mode of attachment and endocytosis.

Current Status in Research and Development

Very little research has been done on the development of vaccines against diseases caused by CP. At present, most studies are focused on methods of diagnosis, information on the cell biology of Chlamydia, and the response of the host to these infections, in an effort to provide a better understanding of the mechanisms of pathogenesis. Recent improvements in isolation techniques have tremendously improved the capacity to detect the organism in clinical specimens. Monoclonal antibodies specific for CP are now commercially available for culture confirmation, and several CP-specific primers have been used in polymerase chain reaction (PCR) detection of organisms. However, efforts to develop a more sensitive multiplex PCR system are in progress. CP does not contain plasmid DNA, and the lack of a genetic shuttle system has hampered progress in the development of molecular biology techniques. However, sequence analysis has identified several genes, including genes encoding structural, functional, and membrane proteins that are similar to those of *C. trachomatis* or *C. psittaci*. Recently, genes encoding heat shock proteins associated with immunopathology or protective responses have been identified in CP. For instance, hsp60 and hsp70 proteins were recognized by using sera from individuals infected with CP. Hsp60 is expressed at high levels during periods of stress and is particularly high in the aberrant form of the organisms. The presence of the molecule is associated with chronic respiratory infections as well as arteriosclerosis. It has also been suggested that much of the tissue injury associated with diseases caused by CP may be the result of the immune response to heat shock proteins.

There is a tremendous gap in the understanding of the host immune responses to infections caused by CP. Cell-mediated immune responses can be demonstrated in individuals infected with CP by blast transformation assays using peripheral blood mononuclear cells. Also, CP induces serum IgM, IgA, and IgG antibody responses. However, the role of cell-mediated or humoral immunity in recovery from infections caused by CP remains to be elucidated. In experimental studies it has been observed that *C. pneumoniae* EB antigens can induce cell-mediated immunity. Recently, a number of species-specific antigens have been characterized. Two of these, an OMP2 and a heat shock protein, have epitope configurations consistent with the capacity to induce a T cell proliferative response. The results of other experiments indicate that immunity to CP may be dependent on the expression of interferon-gamma, a characteristic product of Th-1 type T cells.

Considerable research has been directed at understanding the association of CP with coronary heart disease. Indeed, morphological as well as microbiological evidence indicating the presence of CP in atheromatous plaques has been obtained using electron microscope studies, immunocytochemical staining, and PCR testing of coronary, carotid, and aortic atheroma. In most studies it is clear that the organisms are more commonly found in diseased than in normal tissue. However, although the studies show an association of CP with atheromatous plaques, the role of CP infection in the progression to atherosclerosis is unclear. A major question, regarding whether CP actually causes the condition or is merely a bystander in the process, still remains to be resolved. Other studies have focused on elucidating the mechanism of pathogenesis. The results of these studies suggest that the initial events may be the colonization of CP in alveolar macrophages. Indeed, macrophages or monocytes are likely to play a key role in the infection, serving as a vehicle for dissemination and responsible for the inflammatory response to infection via the elaboration of a variety of cytokines. Recent studies show that CP can grow in a monocyte cell line and can induce the expression of cytokines such as tumor necrosis factor and interleukin-6 as well as increase the expression of the CD14 molecule. In addition to the release of cytokines from macrophages, there is production of cytokines from activated T cells that cause infiltration of monocytes and lymphocytes from the blood. However, it is not clear how these events lead to the development of atherosclerosis.

Much less is known about the microbial components of CP that may serve as vaccine targets. Studies show that the major outer membrane protein (MOMP) of *C. trachomatis* induces the activation of T cells that are protective in infections against *C. trachomatis*. There have been conflicting reports, on the other hand, regarding the immunogenicity of the MOMP of CP. Some studies indicate that the antigen is poorly immunogenic, whereas other studies show a moderate to high immunogenicity. Clearly this area of research needs to be investigated further using purified *C. pneumoniae* MOMP.

There are three experimental animals available for CP infections, namely, mouse, rabbit, and monkey. Mice have been shown to be the most susceptible via intravenous, subcutaneous, or intracerebral infection. These experimental animal models can be used to examine potential vaccine candidates. For example, although CP is primarily a respiratory pathogen, it is conceivable that vaccine administration may prevent systemic spread to other organs. In an effort to understand latent infection caused by CP,

Grayston and his colleagues recently reported that CP lung infection in mice can be reactivated by treatment with cortisone; however, the underlying mechanisms remain to be clarified. In addition, because of the recent association of CP and atherosclerosis, CP models with atherosclerotic complications should be established.

Recent Accomplishments and Development

Although *C. pneumoniae* is a well-known causative agent of respiratory infections, it has also been recently associated with cardiovascular disease. Seroepidemiological studies show the presence of organisms within atherosclerotic lesions. There is also evidence of increased antibody titers against *C. pneumoniae* in individuals with acute myocardial infarction relative to controls. Several other studies have supported these findings. There are studies in progress to determine how *C. pneumoniae* organisms colonize and destroy the walls of blood vessels. In this regard, it is possible that the administration of antimicrobial agents may decrease the risk of cardiovascular disease. Long-term goals should also be directed at examining the effects of CP vaccines on cardiovascular disease.

Future Steps/Challenges

Efforts should be made to obtain improved and more rapid diagnostic methods to ensure timely detection of CP. Studies should be done with more sensitive assays to obtain a better understanding of the epidemiology of diseases caused by CP. Important risk groups should be defined since immunization recommendations will depend on who is at risk. Studies should be conducted to obtain information on the cell biology and molecular genetics of the organism, to characterize CP-specific proteins and identify microbial components that may serve as vaccine targets. The genome of CP should be sequenced. Molecular mechanisms associated with attachment and invasion should be defined, and the host defense mechanisms, strategies for immune evasion, as well as the underlying mechanisms of protection should be elucidated. Major efforts should be made to develop vaccines against infections caused by CP. It is also necessary to develop appropriate animal models that could be useful in investigating chronic or latent CP infections. Specifically, basic research studies should be conducted to determine which factors contribute to the development of atherosclerosis. Further, experiments should be done to evaluate antibiotic treatment on CP-associated coronary heart disease and how such treatment affects mortality associated with CP infection.

Source

Jackson LA, Campbell LA, Schmidt RA, Kuo C, Cappuccio AL, Lee MJ, Grayston JT. Specificity of detection of *Chlamydia pneumoniae* in cardiovascular atheroma: Evaluation of the innocent bystander hypothesis. *Amer J Path* 1997; 50:1785-1790.

Diphtheria and Tetanus

The current diphtheria and tetanus toxoid vaccines are based on technology developed more than 70 years ago. The use of formaldehyde to detoxify tetanus and diphtheria toxins has provided extremely effective vaccines to the point that only a handful of cases of diphtheria and tetanus are reported each year in the United States. However, the processes used in preparing these vaccines are relatively crude and, as a result, both products retain reactogenic properties. With the advent of acellular pertussis vaccines and the increased interest in improving vaccines and immunization programs for adults, work is now under way to develop purified improved diphtheria and tetanus vaccines that can be administered safely to both infants and adults.

One area that has shown great promise during the past few years is the development of a *Salmonella typhi*-based live vector DTP vaccine that can be administered by mucosal (oral or intranasal) immunization with a single dose. Building on work that has resulted in a genetically engineered attenuated strain of *S. typhi,* researchers plan to further engineer this strain (CVD 908-*htr*A) and introduce genes that express protective antigens of *Corynebacterium diphtheriae, Clostridium tetani,* and *Bordetella pertussis,* the pathogenic microorganisms that cause diphtheria, tetanus, and pertussis, respectively. In this way, the *S. typhi* live vector equipped with these genes would express and present these foreign antigens to the human immune system and lead to a protective immune response. It should be noted that protective immunity against tetanus and diphtheria is mediated by serum antitoxin antibodies and precise protective levels (so-called thresholds) of such antibodies have been defined. Thus, using the live vector strategy, it is proposed that mucosal (oral or perhaps intranasal) immunization would elicit protective levels of long-lived serum antibodies.

To stimulate tetanus antitoxin, fragment C (the carboxyl terminus) of tetanus toxin has been selected as the most promising antigen. Fragment C is nontoxic and the antibodies it elicits prevent wild-type toxin from binding to tissue cells. Binding of eukaryotic cells represents a critical preliminary step in the causation of symptoms. Because the toxin exerts its effects intracellularly, if the toxin is prevented from gaining access to cells, it is then unable to initiate harmful effects.

Plasmids have been constructed in which the gene encoding fragment C expression is controlled by different promoters. Mice immunized intranasally with two spaced doses of attenuated *S. typhi* strains CVD 908 or CVD 908-*htr*A expressing fragment C developed serum antitoxin (titers >1:10,000 by ELISA) after just a single dose. Following a booster dose, the serum titers exceeded 1:100,000. In this system using the best construct, serum antitoxin levels exceeded the protective threshold for humans by 72-fold. Preliminary clinical trials are just now beginning with these prototype constructs.

Investigators have also engineered a mutant diphtheria toxin that no longer exhibits toxic activity. This mutant toxin is readily expressed by *S. typhi* and retains the ability to elicit serum antitoxin in mice following intranasal immunization. An additional mutation in the toxin molecule is being introduced to add further to the safety of the diphtheria protein molecule.

Ultimately, a mixture of CVD 908-*htr*A strains is envisioned that can be mixed and administered as a multivalent vaccine. The CVD 908-*htr*A could be expressed just as a single antigen, whereas in other cases, it would simultaneously express several antigens. It is anticipated that over the next several years “proof-of-principle” clinical trials will test the feasibility of a DTP vaccine that can be administered by mucosal immunization.

Work continues on the characterization of the diphtheria (DT) receptor. In 1992, the DT sensitivity gene was cloned by expression cloning and shown to encode the DT receptor. Studies have demonstrated that the cytoplasmic domain of the DT receptor is not necessary for internalization, suggesting that this domain lacks internalization signal(s) found in many receptors that internalize ligands by receptor-mediated endocytosis. Related to this observation was the demonstration that the rate of internalization of the receptor is slow compared to such other classical receptors as the low density lipoprotein receptor, the transferrin receptor, and the lysosomal acid phosphatase precursor. Nevertheless, the cells bearing DT receptors are killed by the toxin since only a single toxin molecule needs to be internalized and the toxin acts enzymatically to inhibit protein synthesis. Teleologically, it seems reasonable that this cell surface protein would be internalized slowly, at a rate similar to that of membrane turnover; the protein is not intended as a ligand receptor, but rather as a cell surface protein to be cleaved into the mature growth factor form and released. An important corollary of this finding of a slow internalization rate is that those laboratories employing nonlethal mutant bacterial toxin molecules in which proteins / peptides are covalently attached (to deliver the latter into the cytosol of an eukaryotic cell) should not use DT, but rather a different toxin (e.g., Pseudomonas exotoxin A) that is internalized by a receptor that normally internalizes ligands in a rapid and efficient manner.

Continued characterization of the toxin-binding domain of the DT receptor has shown that a mature growth factor corresponding to amino acid residues 63 to 148 of the heparin-binding EGF-like growth factor (proHB-EGF) prevented binding of labeled DT to cells by itself binding to the toxin. Employing cells bearing monkey / mouse chimeric proHB-EGF molecules, investigators have been able to narrow the toxin-binding domain to amino acid residues 121 to 148. Studies of site-directed mutagenesis in which specific amino acid residues constitute the toxin-binding site are currently in progress. Information from these studies may be used in the design of peptides to be used as vaccine antigens since antibodies against the toxin-binding site should prevent toxin binding and be protective. Alternatively, and probably of greater practicality, mature HB-EGF, or smaller peptides containing the toxin-binding site, could be used as a modern antidote in cases of diagnosed diphtherial disease instead of horse antidiphtheria toxoid serum, which has a number of known deleterious side effects.

Recent studies have offered some interesting results on the use of anthrax toxin as a vehicle to deliver cytotoxic T lymphocyte (CTL) epitopes *in vivo*. Although *Bacillus anthracis* is not ordinarily included in the list of respiratory pathogens, a detailed understanding of the structure and mode of action of anthrax toxin has led to its potential use as the basis of a new class of vaccines that stimulate a CTL response, as opposed to an antibody response. This is based on the ability of anthrax toxin (and, in principle, other intracellularly acting toxins such as diphtheria) to deliver its enzymatic moieties to the cytosolic compartment of mammalian cells. It is possible to adapt it to deliver heterologous proteins and peptides to the same compartment and permit them to be processed to peptides that are presented by MHC class I molecules at the cell surface.

Most vaccines consist of killed or crippled microbes that are injected into the body, where they trigger the immune system to produce antibodies. But one way that HIV, herpesvirus, and certain other pathogens elude the immune system is by ducking inside immune cells themselves. Researchers have sought to develop vaccines that can teach the immune system's killer T cells to destroy such infected cells. To do so, the vaccine must gain entry to immune cells such as macrophages that display fragments of the pathogen. These antigen-presenting cells, in turn, activate any killer T cells that can recognize the fragments and attack infected cells.

A recent report described the induction of a CTL response in mice against *Listeria monocytogenes* by using a modified, nontoxic form of anthrax toxin to deliver a known CTL epitope (a 9-residue peptide) from listeriolysin O. Additional studies have succeeded in delivering epitopes and inducing a protective CTL response against lymphocytic choriomeningitis virus in mice. Success with both bacterial and viral systems offers the promise of a general delivery vehicle that may serve as the basis for CTL-based vaccines against a variety of human maladies, including persistent viruses (HIV, hepatitis B), other diseases caused by intracellular pathogens, and perhaps even certain types of cancer. Some respiratory ailments would certainly fall under this umbrella (e.g., respiratory syncytial virus infection).

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Group A Streptococci (GAS)

Group A streptococci cause an estimated 25 million infections each year in the United States. These infections occur primarily in school age children and present as upper respiratory tract or skin infections. Antibiotic therapy is usually effective in eliminating the infection. However, these bacteria have the potential to initiate

life-threatening infections such as rheumatic fever, rheumatic heart disease, or acute glomerulonephritis. Acute rheumatic fever occurs in all parts of the world and is the major cause of heart disease in children in developing countries. Rates of rheumatic heart disease range from 0.2 to 0.5 per 100,000 in affluent, developed communities to 125 to 960 per 100,000 in segregated, low socioeconomic populations.

Although the incidence of rheumatic heart disease in the United States and other developed countries declined markedly during the late 20th century, the 1980s witnessed a resurgence in serious GAS infections, often with complications that included severe soft-tissue invasion and toxic shock-like syndromes among otherwise healthy individuals. One theory for the increase in severe GAS infections is the emergence of a more virulent strain, which is supported by the isolation of a GAS strain with a more invasive phenotype. However, a number of studies indicate that the same GAS strain causes uncomplicated pharyngitis and severe invasive GAS disease.

Current research studies are focused on elucidating the mechanism of invasion, the host factors that may determine severity of GAS disease, and virulence factors involved in the pathogenesis of GAS infections. In addition, studies on the use and efficacy of antimicrobial agents for controlling GAS infections have become focused on macrolide antibiotics because erythromycin-resistant GAS have been isolated in several parts of the world. This is a major concern because erythromycin has been the drug of choice for treating streptococcal infections in patients allergic to penicillin. These factors emphasize the need for an efficacious vaccine.

The most significant obstacle to developing a vaccine against GAS is circumventing an autoimmune response due to the immunological cross-reactivity between epitopes on the streptococcal cell and human tissues (heart, brain, and joints). All clinical isolates of GAS produce immunogenic surface proteins that have a role in the ability of the bacteria to colonize the host and cause disease. Streptococcal M protein is one of the surface molecules that was first described as being important in pathogenesis; it is responsible for resistance of GAS to phagocytosis and is a major virulence factor.

There is a family of M proteins that have several shared structural properties among the GAS. Although the amino terminus of the M protein is hypervariable, containing type-specific nucleic acid sequences, the carboxyl terminus is conserved among the different M proteins. More than 92 different types of M proteins have been identified. Although antibodies against M protein have been shown to mediate type-specific protective immunity, specific M protein epitopes have been shown to cross-react with proteins of the host (e.g., myosin, laminin). It may be possible to eliminate this cross-reactivity by constructing vaccine candidates with only specific epitopes of M protein that are thought to be protective.

Currently, NIAID is supporting research aimed at identifying protective epitopes of M proteins from strains of GAS. Animal models are being used to assess the production of opsonizing and non-cross-reacting antibodies elicited after exposure to vaccines under development. An immunofluorescence assay using human heart tissue is one of the methodologies employed to test for heart cross-reactive antibodies. In addition, a number of different approaches to vaccine development are being funded that are based on other streptococcal proteins associated with virulence of GAS (i.e., c5a peptidase and cysteine protease).

Strategies for vaccine development have included immunization at parenteral and intranasal sites. The parenteral route has been used because it is well recognized that protective immunity to GAS infection can be achieved with serum-derived IgG directed to type-specific immunodeterminants of the M protein. The intranasal route has been used because early studies indicated the importance of local immune factors. Pharyngeal challenge studies had been performed following immunization with a highly purified, acid-extracted M protein. Individuals immunized at the intranasal site had lower rates of nasopharyngeal colonization and clinical illness as compared to individuals immunized subcutaneously who exhibited a decrease in clinical illness only and had no change in the level of nasopharyngeal colonization.

Several vaccine candidates have been developed using synthetic or recombinant peptide fragments derived from type-specific sequences of the M protein. A number of recombinant, multivalent M protein vaccines have been constructed by placing protective peptide fragments of various M genes in tandem. Research studies indicated that the response to specific epitopes in hybrid M proteins depends on the size and location of the subunits, which were important considerations for constructing these vaccine candidates. When tested in rabbits using a parenteral route, an octavalent M protein vaccine evoked a broadly protective immune response as measured by opsonic antibodies with bactericidal activity, and none of the antisera reacted with human heart tissue.

In a recent study, protective systemic immune responses were evoked in mice following intranasal administration of a recombinant protein consisting of streptococcal M protein fragments fused to a toxin subunit; 2 of 18 immunized mice died of infection following intraperitonal challenge with a virulent GAS as compared to 15 of 20 immunized with the toxin subunit. In addition, immunogenicity studies in mice demonstrated that significant levels of serum antibodies and detectable levels of salivary IgA were produced.

An issue to be addressed is the multiplicity of M serotypes expressed by GAS. Thus, an important consideration is the selection of M serotypes to be included in a vaccine; that will be guided by epidemiologic studies and will focus on those serotypes that are associated with rheumatic fever, cause serious disease, or are frequent causes of uncomplicated infections. It may be necessary to develop several multivalent proteins that contain protective epitopes that could be mixed according to geographic differences in serotype prevalence.

An alternative approach has been to use the highly conserved region of the carboxyl terminus of the M protein as a target for protective antibodies. With regard to safety, human tissue cross-reactive epitopes identified to date have been located in the nonconserved regions of the M proteins. In addition, most adults living in areas where exposure to streptococci is high have antibodies to peptides in the conserved region. Research studies have demonstrated that immunization of mice by intranasal administration of synthetic peptides, corresponding to conserved epitopes of M protein, conjugated to cholera toxin B subunit induced both homologous and heterologous protection against colonization and disease following intranasal and oral challenge. These data were used to develop a live vector to evoke a GAS-specific mucosal immune response.

Although live-vector antigen delivery approaches have been used to develop vaccines, most vectors have been pathogens that require attenuation before cloning heterologous nucleic acid sequences. The use of a nonpathogenic commensal organism circumvents many of the problems involving appropriate attenuation while retaining invasive properties. The conserved region of the M6 protein was cloned and expressed on the surface of *Streptococcus gordonii*, a commensal organism of the oral cavity. Both IgA and IgG antibodies were produced in mice following oral and intranasal administration of this vaccine candidate. Similar studies in rabbits using the same routes to administer the vaccine evoked M-specific IgG and IgA. In addition, there was no reactivity to human heart tissue when serum antibodies from rabbits colonized for 11 weeks were tested. An issue to be addressed is the unknown long-term effects of colonization with an engineered oral commensal expressing a conserved epitope of GAS M protein. Colonization followed by eradication of the vaccine strain would be an appropriate cautious approach for initial safety and immunogenicity studies.

Another GAS vaccine candidate based on the conserved region of the M protein is being developed. Overlapping synthetic peptides spanning the conserved region of the M5 protein were tested with serum from adult Aborigines and Thais living in areas where exposure to streptococci is very high. Because high levels of antibody specific for peptide 145 were detected in these individuals, this peptide was chosen for further evaluation. In preliminary studies mice immunized by the subcutaneous route with peptide 145 emulsified in complete Freund's adjuvant produced antibodies that opsonized streptococci of different M types. Work in progress includes the evaluation of different adjuvants and challenge studies with virulent GAS.

C5a peptidase is another surface-bound protein that has been postulated as a virulence factor of GAS. Immunization with C5a peptidase is based on studies in mice demonstrating that C5a peptidase is required for nasopharyngeal colonization. Thus neutralizing antibodies against the C5a peptidase may provide protection against streptococcal colonization. Recent studies have shown that intranasal immunization of mice and rabbits with an affinity-purified C5a peptidase from an M49 strain expressed in *E. coli* induced an immune response. In the rabbit model, high titers of antibody were produced that neutralized peptidase activity *in vitro* against C5a peptidase from M49 as well as C5a peptidase associated with serotypes M1, M6, and M12. In the mouse model, high titers of serum IgG and a significant increase in salivary IgA were produced, as compared to control mice immunized with phosphate-buffered saline (PBS). Enhanced clearance of GAS from the nasopharynx in immunized mice was demonstrated when the M49 strain was used to challenge mice; 1 of 13 immunized mice was culture positive for GAS 10 days after vaccination in contrast to 30 to 58 percent of unvaccinated controls that remained culture positive for 6 days. In addition, immunized mice were protected against serotype M2, M11, M1, and M6 GAS pharyngeal colonization, as indicated by statistically significant differences in the number of mice colonized with the different serotypes. These preliminary studies suggest that a C5a peptidase has potential as a vaccine candidate directed at preventing streptococcal pharyngitis by interfering with GAS colonization.

Studies on the effect of vaccination with the extracellular cysteine protease from GAS have shown significantly enhanced survival of mice when challenged with a lethal dose of virulent *Streptococcus pyogenes* following passive immunization with rabbit antiserum directed against purified cysteine protease; none of the control mice inoculated with PBS survived beyond 40 hours as compared to approximately 87 percent survival of passively immunized mice at 40 hours and 42 percent at 72 hours. In addition, mice actively immunized with the cysteine protease had a significantly longer time to death than the control group; none of the mice inoculated with PBS survived beyond 30 hours while approximately 66 percent of actively immunized survived at 30 hours and approximately 22 percent at 50 hours. Studies are currently being conducted to examine the contribution of local versus systemic immune responses to the protease in providing protection against lethal challenge.

As described above, a number of different approaches have led to the development of several promising vaccine candidates. Depending on disease manifestation and geographic location, a combination of vaccines may be needed for control and prevention of GAS disease and sequelae (i.e., rheumatic heart disease). A GAS vaccine for prevention of streptococcal pharyngitis would need to evoke a mucosal immune response aimed at preventing colonization of GAS. However, a vaccine for prevention of rheumatic heart disease, streptococcal toxic shock, and necrotizing fasciitis would need to evoke circulating opsonizing antibodies. Safety issues involving human heart cross-reactive antibodies can now be addressed by using recombinant DNA techniques to select potentially protective epitopes to be included in vaccine candidates and thereby exclude epitopes associated with cross-reactivity.

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Group B Streptococci (GBS)

Infections caused by group B streptococci are the leading cause of bacterial disease and death in newborn infants as well as a frequent cause of disease in peripartum women and in adults with chronic medical conditions. In the past 5 years, significant progress has been made in the development and clinical testing of vaccines to prevent GBS infections in newborn infants, although all risk groups may benefit from a preventive vaccine. Different types of GBS have been identified by the variations in the composition and structure of their capsular polysaccharide (PS). GBS types Ia, Ib, II, III, and V can all cause disease. Antibodies generated against the capsular PS have been shown to be protective.

In the United States, neonatal disease prevention strategies are currently focused on the identification of vaginal and rectal GBS colonization in pregnant women and the use of antibiotics during labor and delivery in those women who are colonized or present with other risk factors. There is early evidence to suggest that this strategy is effective, but it has not been able to totally eliminate GBS disease and, as a consequence, has encouraged the widespread use of antibiotics. Active immunization of women during the third trimester of pregnancy, to induce antibodies and passively protect their newborns, has great potential for the prevention of both maternal and infant disease and does not rely on detection of colonization. It is also possible that immunization of childbearing-aged women may affect their subsequent colonization with GBS.

In the 1980s, a type III GBS polysaccharide vaccine was evaluated in a small group of third-trimester pregnant women. The vaccine was safe but not universally immunogenic, with only 63 percent of nonimmune women responding to the vaccine. To improve immunogenicity, as was the case with *Haemophilus influenzae* type b, GBS polysaccharides were conjugated with protein components. Several type Ia, Ib, II, III, and V polysaccharide-protein conjugate vaccines have been prepared and evaluated in a series of phase I/II clinical trials. All of the conjugate vaccines have been found to be safe, nonreactogenic, and immunogenic. The GBS type III-tetanus toxoid conjugate vaccine was evaluated in phase I and II clinical trials in childbearing-aged women. The vaccine was found to be safe and significantly more immunogenic than the uncoupled PS vaccine. Antibodies evoked by the conjugate vaccine recognized a conformationally dependent epitope of the type III capsular polysaccharide and promoted opsonophagocytosis and killing of GBS. In a mouse model, maternal immunization with the conjugate vaccine protected the neonatal offspring from lethal challenge with type III GBS.

The National Institute of Allergy and Infectious Diseases has supported the production of a larger lot of serotype III-tetanus toxoid conjugate vaccine for clinical evaluation as a potential maternal immunogen. Ongoing research activities are focused on the use of alternative protein carriers and on the use of alum-based adjuvants to enhance immunogenicity with decreasing concentrations of the polysaccharide and protein components.

The widespread use of a successful multivalent GBS conjugate vaccine could significantly reduce the morbidity and mortality associated with this major neonatal pathogen. Consequently, one of the goals of the NIAID GBS program is a phase III vaccine efficacy trial for the prevention of GBS disease. Before this trial can be initiated, considerable work needs to be done on the epidemiology and pathogenesis of the disease, the basic biology of GBS, and the further development of immunogenic vaccine constructs. To facilitate the resolution of these and other relevant issues, NIAID awarded a research and development contract, in 1992, to the Brigham and Women's Hospital. The contract provided support for collaborative as well as multifaceted clinical and basic research efforts on group B streptococcal disease. This contract was recompeted in 1997, and a new award was made to the Brigham and Women's Hospital. The contract workscope now includes a focus on the natural history of GBS colonization of women and on the role of GBS as a pathogen in those with underlying chronic disease.

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Haemophilus influenzae type b (Hib)

Before the availability of effective vaccines, 16,000 to 25,000 children in the United States developed infections caused by Hib each year. Bacterial meningitis, the most serious complication of Hib disease, occurred in 60 percent of affected children. Ten percent of these children died, while many survivors suffered serious and permanent disabilities. Other infections included bacteremia, pneumonia, empyema, pericarditis, cellulitis, septic arthritis, and epiglottitis. From 1988 to 1991, following licensure of Hib conjugate vaccines for older infants and then for young infants, there were significant reductions in the incidence of Hib invasive disease and related morbidity and mortality. In the past few years, Hib disease has decreased by 95 percent, and the Centers for Disease Control and Prevention included Hib disease among children under 5 years of age as one of the vaccine-preventable diseases targeted for elimination from the United States by 1996. As of July 1997, this goal is close but not yet achieved. It should be noted that decreased incidence of Hib disease began before the vaccine was widely used in infants under 18 months of age. This decrease appears to be due to the fact that the Hib conjugate vaccine also decreased the asymptomatic carriage rate in older children, thereby reducing the risk of infection in infants under 18 months of age. The magnitude of this "herd immunity" effect was generally unanticipated and has led to research and prevention avenues that may show promise for other infections caused by encapsulated bacteria.

The capsular polysaccharide antigens of Hib and other encapsulated bacteria are important determinants of virulence and hence have been targeted for vaccine development. Antibodies specific for these polysaccharides are known to confer protection against invasive disease. Although polysaccharide antigens are not usually immunogenic in infants, their immunogenicity can be increased greatly by covalently coupling them to a carrier protein. In the resulting polysaccharide-protein conjugate, thymic-dependent features are conferred on the antibody response to the poly-

saccharide, thereby influencing the amount and type of antibody generated after reimmunization with the conjugate. In recent years, such conjugate vaccines have been used most extensively to immunize infants against Hib invasive disease.

In 1990, following extensive clinical testing, two Hib conjugate vaccines were licensed (HibTITER®, made by Lederle/Praxis Biologicals, Inc., and PedvaxHib®, made by Merck, Sharp & Dohme and Co.) for use in infants, beginning at 2 months of age. A third Hib conjugate vaccine, PRP-T (Pasteur-Merieux), was subsequently licensed in the United States for use in young infants, beginning at 2 months of age. These conjugate vaccines represent a major advance in the prevention of meningitis in infants and lend impetus to the development of other polysaccharide-protein conjugate vaccines for use in infants.

Hib conjugate vaccines differ with respect to the molecular size of their polysaccharide component, the particular protein used as carrier, and the type of linkage between the polysaccharide and protein. It is not surprising that such structural differences result in vaccines with different immunologic properties. Reported findings suggest fundamental differences between conjugate vaccines in their ability to induce protective immunity, as well as in the quality (avidity) of the antibody produced. Hib conjugate vaccines are generally administered at the same time as the DTP (diphtheria, tetanus toxoid, and pertussis vaccine, adsorbed) vaccine. Attempts to combine all of these vaccine preparations into one, to reduce the number of injections an infant would receive during the primary immunization schedule, have been a major focus in recent years. The first combination vaccine (Tetramune, made by Wyeth-Lederle Vaccines and Pediatrics), composed of a Hib conjugate and DTP, was licensed in April 1993.

Detailed studies on the immune response of humans of different ages to various Hib-conjugate vaccines have increased the understanding of the ontogeny (development) of the immune response to polysaccharide antigens, as well as the influence of the form in which antigen is presented (T cell-dependent vs. T cell-independent) on the type (immunoglobulin class) of antibody response generated. Polysaccharide-protein conjugate vaccines induce predominantly an IgG1 subclass antibody response. Reimmunization of infants with isolated polysaccharide after priming with a polysaccharide-protein conjugate increases the amount of both previously expressed and new antibody clonotypes produced. Since the new clonotypes made tend to be of the IgG2 subclass, there appear to be relative differences in the IgG subclass of antibody generated in response to different forms of the antigen. Researchers are investigating the hypothesis that IgG1 may be the predominant subclass induced by immunization with T-dependent antigens, whereas T-independent antigens may evoke predominantly IgG2 responses. It is not yet clear whether the predominance of the IgG1 responses in infants after primary immunization with the conjugate reflects the inability to activate B cell subsets specific for IgG2 or the lack of isotype switching to IgG2 by activated B cells.

Work is continuing on the possibility of using other Hib antigens in vaccines. Several surface-exposed proteins and structures such as fimbriae/pili are capable of stimulating protective antibodies. Some of these surface proteins are conserved among Hib strains, while others show considerable strain-to-strain variation. Investigators have found that pili on the surface of Hib facilitate adherence of these bacteria to mucosal epithelial cells. Fimbriae are found on all strains of Hib and, although minor structural differences have been reported, all appear to share a common immunogenic peptide that is recognized by antibody. Such a peptide might be regarded as an important component of a vaccine against *H. influenzae*. It would stimulate the production of antibodies that interfere with adherence of the bacterium to airway tissues; such adherence is essential for invasiveness and the subsequent development of disease in infants. Investigations have also focused on iron-related phenotypes of *H. influenzae*. Several laboratories have identified which iron-containing human proteins are potential sources of iron for *H. influenzae*. Acquisition of iron from these protein sources involves a bacterial protein receptor. Since this protein receptor is on the outer membrane of the bacterium, antibodies directed against this receptor might also facilitate the development of protective immunity.

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Nontypeable *Haemophilus influenzae*

Nontypeable *Haemophilus influenzae* (NTHi) frequently causes recurrent infections of the respiratory tract in humans and normally resides in the nasopharynx. The organism is an important human pathogen in several settings and is most often associated with otitis media and sinusitis. NTHi is consistently a major cause of otitis media in infants and children and is responsible for approximately one-quarter to one-third of all episodes. Otitis media represents an enormous national health problem both from the point of view of human suffering and of cost. Approximately 80 percent of children will have had at least one episode of otitis media by 3 years of age. Otitis media is the most common reason for visits to pediatricians, and the annual cost of medical care for this disease nationally is estimated to be $2 billion. Serologic studies and studies of the effect of antibiotics indicate that NTHi is an important cause of lower respiratory tract infections in patients with chronic obstructive pulmonary disease (COPD). COPD is the fifth leading cause of death in the United States, with infections being the major contributing factor. Recent studies have implicated *H. influenzae* as a common cause of bacterial pneumonia in

patients with AIDS. Carefully performed studies in Papua New Guinea, Hong Kong, Pakistan, and The Gambia have demonstrated the importance of NTHi as a common cause of lower respiratory tract infections in children, accounting for a significant fraction of the more than 5 million deaths in this population annually. Neonatal sepsis caused by NTHi has been recognized with increasing frequency during the past decade. The infection is associated with a 50 percent mortality overall and a 90 percent mortality among premature infants. Despite this burden of disease, little is known about the pathogenesis of this infection.

All strains of *H. influenzae* share the metabolic requirement for heme, an iron-containing compound, for growth. Since *H. influenzae* cannot make its own heme, it must acquire heme from its human host to grow in the body and cause an infection. Although the body contains large amounts of heme, the heme is not readily available since it is tightly bound either to hemoglobin within red blood cells or to other proteins. To grow and cause an infection in the body, *H. influenzae* has evolved specific mechanisms that allow this bacterial pathogen to "steal" heme from the human proteins for use in its metabolic pathways.

Recent research has uncovered a novel *H. influenzae* protein involved in the acquisition of heme from the human host. Nontypeable *H. influenzae* produces a protein, designated HxuA, that is required for *H. influenzae* to be able to obtain heme from a certain human heme-binding protein. The HxuA protein of *H. influenzae* is secreted from the organism and binds to heme:hemopexin, a human protein, one of the most important sources of heme in the human body. *H. influenzae* cannot steal the heme from heme:hemopexin unless the organism can make the HxuA protein. This HxuA protein traps the heme:hemopexin protein and brings it back to the *H. influenzae* cell so that this bacterium can utilize heme bound to the hemopexin, thus enabling itself to grow and cause disease.

Clarifying this essential role of HxuA protein in obtaining heme from heme:hemopexin has led to the consideration of HxuA as a possible vaccine candidate for the prevention of disease caused by nontypeable *H. influenzae*. Vaccination with HxuA protein may be able to induce the synthesis of antibodies that will bind to the HxuA protein when it is secreted from *H. influenzae* and, thereby, prevent *H. influenzae* from obtaining heme from heme:hemopexin. This inhibition of the ability of *H. influenzae* to steal heme from its human host may kill *H. influenzae* or otherwise prevent *H. influenzae* from growing and causing disease. Testing of the ability of HxuA to induce the synthesis of antibodies protective against nontypeable *H. influenzae* in an animal model is in progress.

A major problem among children is the recurrence of middle ear infections by *H. influenzae* organisms. NIAID-supported research has revealed the mechanism of recurrent infections in this setting. Studies of the outer membrane proteins have revealed that the surface characteristics of the bacterium allow for these recurrent infections. Animal models have demonstrated that following an episode of otitis media the immune system makes antibodies to one specific region of one specific molecule (loop 5 of the outer membrane protein P2). These studies revealed that the bacterium induces the host to mount an immune response to a specific portion of the protein that is extremely heterogeneous among strains. Therefore, the immune response is effective at clearing the bacterium from the middle ear, but only for that particular strain that caused the infection. Given this understanding, it appears that recurrent otitis media can be better understood as a child would remain susceptible to other strains of *H. influenzae* that have different protein sequences in the loop 5 region. This observation has important implications in the design of vaccines to prevent nontypeable *H. influenzae* infections. Additional work has identified another molecule (P6) that does not show sequence differences among strains. This P6 outer membrane protein has many unique characteristics, suggesting that it will be an effective vaccine antigen. Initial clinical trials are about to begin to test the safety and immunogenicity of P6.

Several other *H. influenzae* surface proteins also have been identified as strong vaccine candidates. A recombinant form of a novel *H. influenzae* outer membrane protein designated Hin47 has been clinically developed by Pasteur Merieux Connaught through a technology license agreement with Antex Biologics Inc. The vaccine, which relies on an adhesin-receptor technology and is combined with an adjuvant, recently began phase I testing in adults to determine its safety and immunogenicity. The ultimate intent is to develop an effective vaccine that will prevent otitis media and its complications in the pediatric population.

Another highly conserved protein associated with both type b and nontypeable *H. influenzae* strains is a 42 kDa membrane lipoprotein referred to as protein D. SmithKline Beecham has been studying this nonacylated form of lipoprotein D as a potential carrier for both its PRP and pneumococcal conjugate vaccines as well as a vaccine for nonencapsulated strains of *H. influenzae*. Preclinical studies in rats have demonstrated high titers of bactericidal antibody against homologous and heterologous *H. influenzae* strains following hyperimmunization. Clinical testing of protein D as a carrier has just recently begun.

Lipooligosaccharide (LOS) has been shown to be a major surface antigen of nontypeable *H. influenzae* capable of eliciting bactericidal and opsonic antibodies in animals. When prepared as a detoxified protein conjugate (i.e., LOS linked to tetanus toxoid or other high molecular weight *H. influenzae* proteins), IgG anti-LOS antibody levels rose significantly in mice and rabbits to the homologous LOS following two or three injections administered subcutaneously or intramuscularly. These results were enhanced by the addition of monophosphoryl lipid A plus trehalose dimycolate.

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Influenza

Among viruses, influenza is notable in its ability to produce annual epidemics of disease in both developed and developing countries. Recorded as pneumonia and influenza (P&I) morbidity and mortality, the annual toll of P&I-related deaths typically ranges from 10,000 to 20,000, with estimates ranging as high as 50,000 during severe outbreaks. Despite vaccination or prior infection, the population susceptibility to infection is renewed annually because influenza viruses undergo two major forms of antigenic variation: antigenic drift and antigenic shift. The sudden appearance of a new antigenic subtype is considered a shift, while a more subtle antigenic variation within a subtype is considered antigenic drift. Consequently, influenza viruses have the inherent capacity to change the antigenic makeup of their surface proteins. If the change is a major one with little or no cross-reactivity to previously circulating strains (i.e., an antigenic shift), serious epidemics can result because of low protective immunity in the population. Such changes also are responsible for variations in virulence, host range, and infectivity of the virus. These "chameleon-like" properties can result in serious epidemics. For example, during the catastrophic epidemic of 1918–19, about 20 million to 40 million people died worldwide, and 500,000 died in the United States (196,000 people during October 1918); it was the worst epidemic the United States has ever experienced.

Influenza disease has long been recognized as a major uncontrolled health problem in the United States. Vaccines continue to be the focus of control for all groups at risk of serious complications of influenza infection. Although annual influenza vaccination rates have risen from 1985 (23 percent) through 1994 (55 percent) for persons aged ≥ 65 years, the estimate of mortality related to pneumonia and influenza remains at 90 percent for that age group. Most vaccine manufacturers and other scientific institutions with interests in influenza vaccines are exploring new ways to improve vaccine performance.

The National Institute of Allergy and Infectious Diseases is engaged in a multifaceted effort to improve the inactivated vaccine that is now licensed for use against influenza. NIAID intramural scientists conducted a series of phase I and II clinical studies in the development of a live, attenuated, cold-adapted (*ca*) influenza virus vaccine initially discovered by Dr. Hunein Maassab of the University of Michigan. The unique characteristic of this attenuated strain is the ability to grow at 25°C and the inability to grow at ≥38°C (temperature sensitivity), allowing the virus to colonize the upper respiratory tract while lacking the ability to infect the warmer temperatures of the lower respiratory tract. The *ca* vaccine presents an alternative vaccine approach that offers several advantages, including stimulating a wider range of antibodies; inducing local, humoral, and cellular immunity; and the ability to administer the vaccine intranasally, at the site of infection. This *ca* vaccine has been studied clinically in a wide range of age and high-risk groups as a monovalent, bivalent, and trivalent preparation.

A Collaborative Research and Development Agreement (CRADA) between NIAID and Aviron exists to complete development of the intranasal *ca* influenza vaccine. This CRADA provides a mechanism that allows for exclusivity of the preclinical and clinical data and the scientific collaboration needed to assist in the clinical development leading to licensure of this vaccine. Since the CRADA agreement was signed over 2 years ago, more than six clinical studies were designed and conducted to test safety, immunogenicity, and efficacy of a trivalent formation with a nasal spray-syringe delivery system. This approach is based on the data from previous NIAID-supported clinical studies that demonstrated safety and immunogenicity using a large-particle spray. On July 14, 1997, the initial results of a phase III efficacy study in more than 1,600 children who received one or two doses indicated that the *ca* influenza vaccine is approximately 93 percent efficacious in preventing culture-confirmed cases of influenza. The results of this efficacy study confirm the results of previous studies that demonstrated that the vaccine is safe, immunogenic, and at least as effective as the inactivated vaccine in preventing culture-confirmed influenza virus illness. Additional Vaccine and Treatment Evaluation Unit multicenter studies are planned during the 1997–98 influenza season with a special emphasis on the pediatric population and high-risk groups.

Previously published results of a 5-year influenza vaccine study estimate efficacy of the inactivated vaccine to range from 69 to 76 percent, depending on the type of influenza virus prevalent that year and the definition of efficacy. Under the same conditions, efficacy for the *ca* vaccine was estimated to range from 32 to 85 percent. However, when the most stringent criterion for efficacy is applied (i.e., identifying influenza virus as the source of the illness), equivalent efficacies were demonstrable for both inactivated and *ca* vaccines.

The similar efficacy noted between the licensed and the experimental vaccine, as well as the ease of administering the new vaccine (nose drop vs. injection), may result in greater coverage and acceptability should the *ca* vaccine be licensed. Phase I clinical trial testing of a monovalent *ca* influenza B vaccine has shown it to be safe and nonreactogenic in all populations tested, including children with mild to moderate asthma. Future studies of the trivalent *ca* vaccine will be targeted for both children and high-risk populations, such as the elderly.

Preliminary data for children who have not been previously exposed to influenza indicate that the *ca* vaccine may be more efficacious than the licensed vaccine in this population. In an effort to evaluate the protective effect of a combination of the *ca* influenza vaccine and the currently licensed, inactivated, trivalent influenza vaccine, a randomized, double-blind, controlled trial in 662 elderly residents (mean age 84.2 years) of long-term health care units was conducted. The trial was designed to compare the protective efficacy of administering both vaccines to the efficacy of the inactivated influenza vaccine alone. Volunteers who received combined vaccination and who were subsequently exposed to influenza A virus had significantly lower rates of influenza A virus infection than those receiving only inactivated vaccine. Although all 3 years of this study were associated with acute—but mild—influenza A virus infections, two cases of influenza A virus infection resulted in pneumonia and hospitalization; one of the two patients died, and both cases occurred in patients given only the inactivated vaccine. These findings suggest a potential adjunct role of *ca* vaccines in the high-risk elderly.

The NIAID has continued efforts to improve the use and effectiveness of the inactivated virus vaccine using several approaches, including the use of adjuvants. In addition, the Institute continues to work with both independent investigators and pharmaceutical companies to evaluate alternative strategies. Previous collabora-

tions include the use of microencapsulation, oral dosing, and liposomes as vaccine delivery systems to improve the immunogenicity of the vaccine. Clinical studies have been conducted using these novel approaches. Although preliminary results indicate that these vaccine delivery systems are safe, the consistency and stability of the product and the issue of efficacy remain as serious obstacles. The use of more conventional adjuvants for improving the immunogenicity of the licensed influenza vaccine also is being evaluated by several vaccine manufacturers. These efforts are supplemented by basic research on the mechanisms involved in the generation of significant mucosal immunity.

Although current efforts to improve influenza vaccines have focused on stimulating the humoral and/or local antibody response, the potential role of peptide-based vaccines in improving cell-mediated immunity is also being considered. Several influenza virus proteins, including the hemagglutinin (HA), a nonstructural protein (NS1), the RNA polymerase protein (PA), and the nucleoprotein (NP) of influenza viruses, are now thought to stimulate cytotoxic T lymphocyte (CTL) responses. A molecular dissection of the amino acid sequences responsible for stimulating CTL responses indicates that they are widely conserved and small in number (i.e., 12 to 15 amino acids for the transmembrane region of the HA). This suggests that such viral proteins may be of value in a CTL-based vaccine strategy, as well as to induce the formation of an array of cross-reactive protective antibodies. Experimental studies using mice support such a view and show that immunization with a hybrid protein containing CTL-specific sequences for an H1N1 virus reduces virus titers in the lungs of mice challenged with either an H1N1 or an H3N2 influenza virus. A baculovirus-expressed, CTL-specific influenza virus protein has also been evaluated in the mouse model and has been shown to be protective against a lethal virus challenge. Additional studies are under way to evaluate further the immunogenicity of this protein.

Over the past several influenza seasons, a few new influenza vaccine candidates were tested in healthy young adults in phase I clinical studies. One vaccine, a recombinant baculovirus-expressed influenza virus HA, appears to be nonreactogenic but capable of eliciting a robust immunological response at high doses. Another was a purified influenza virus (N2) neuraminidase component vaccine. Clinical studies conducted during the past 2 years included a dose-range clinical study in the young adult and high-risk elderly population. Preliminary results indicate that these vaccines are safe and immunogenic, in adults, at several test doses considered. Additional clinical studies are planned for the 1997–98 influenza season.

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Measles, Mumps, and Rubella

These three childhood diseases are now rare in the United States but not in the developing world, where measles still kills 1 million children each year. The reemergence of measles in 1989–92 in the United States stimulated a rededication of efforts to control and eventually eradicate measles. The World Health Organization (WHO) has stated that after polio, the next disease targeted to be eradicated is measles, and has targeted 2010 as the measles eradication date. In some parts of the world, the most popular vaccine used for measles eradication efforts is the MMR (measles, mumps, and rubella) vaccine. Since mumps and rubella viruses appear to have no natural reservoir, they, like measles, potentially could be eradicated. The possibility of nationally, regionally, or globally eradicating all three diseases during the same time has recently been considered. However, as reviewed below, unlike measles, little scientific attention is being focused on mumps and rubella.

Measles

Between 1981 and 1988, a steady average of 3,000 cases of measles occurred in the United States each year. This rate was a reduction of over 99 percent from the 400,000 to 700,000 annual cases reported before the introduction of a vaccine in 1963. However, in the early part of this decade, a resurgence of measles occurred in the United States. From 1989 to 1991, 55,165 cases with 123 deaths were reported. The major cause of the reemergence of measles in the United States was the failure to vaccinate children at the appropriate age rather than failure of vaccine efficacy. The United States undertook a major effort to increase vaccine coverage, and the number of cases in 1996 was reduced to 488. Many of these cases were imported cases, and in the past few years, there have been periods when indigenous measles cases were not reported and transmission of the virus appears to have been interrupted.

Worldwide, measles reporting is incomplete, but in 1996, the disease burden was estimated at 36.5 million measles cases and 1 million deaths. Measles remains a major health problem accounting for 10 percent of global mortality from all causes among children aged less than 5 years. There is substantial underreporting of

official measles cases, but the number of cases of measles officially reported to the WHO has dropped from 1,356,992 in 1990 to 797,322 in 1996. The majority (445,949) of these cases are in the African region. Of the remaining cases, the European region accounted for 162,967 cases, the Western Pacific region for 84,459, the Southeast Asia region for 81,477, the Eastern Mediterranean region for 20,361, and the American region for 2,109. This represented the lowest number of cases ever reported from the Americas. Forty countries reported no measles cases in 1996.

Measles vaccine coverage worldwide has gone from 5 percent in 1977, to 16 percent in 1983, to 81 percent in 1996. The success of the polio eradication campaign and the success in reducing measles in the Americas have led to a global call for increased efforts to control measles worldwide. The 1990 World Summit for Children adopted a goal of vaccinating 90 percent of children against measles by the year 2000, and in 1994, the Pan American Sanitary Conference resolved to eliminate measles from the Western Hemisphere by the year 2000. To accomplish these objectives, measles control has incorporated lessons learned from the polio eradication campaign. The Pan American Health Organization documented that the last case of paralytic poliomyelitis with a wild-virus isolate in the Western Hemisphere occurred in Peru on August 23, 1991. The successful methods developed during this pioneering regional eradication effort led to a now-standard worldwide polio eradication strategy of (1) achieving and maintaining high routine vaccine coverage; (2) giving supplemental vaccine doses during National Immunization Days (NID) to interrupt wild-virus transmission; (3) conducting mopping-up immunization campaigns, and (4) developing sensitive systems for surveillance. For measles, this approach has been termed the keep-up, catch-up, follow-up program, and it has been extremely successful in many countries, particularly in South America. However, some countries have used other control strategies, and the U.S. experience with a two-dose immunization schedule demonstrates that maintenance of high levels of routine immunization also can lead to successful interruption of virus transmission.

Unfortunately, two recent reemergences of measles have illustrated that many challenges remain for measles elimination programs. In 1997, despite a well-coordinated measles control program, measles reemerged in Brazil. By the middle of the year, Sao Paulo state reported more than 400 cases, after having virtually eliminated measles for the previous 6 years. In 1997 in Canada, despite a successful change from a one-dose to two-dose schedule and extensive catch-up campaigns, measles reemerged. An epidemic started after importation of measles into a university setting and spread within the British Columbia Province and later to Alberta. By midyear, more than 500 cases had been reported. These epidemics are currently being studied to understand their cause and to "fine-tune" measles control strategies.

As a public health tool, the current vaccine has some deficiencies. It has a primary vaccine failure rate of about 5 percent, and thus susceptible individuals accumulate in the population. This failure rate is higher if the current vaccine is given at less than 12 to 15 months of age, when maternal antibody interferes with vaccine efficacy. In developing countries, where measles continues to claim more than 1 million lives each year, infants are at greatest risk for serious disease and complications during the interval between loss of maternal antibody and receipt of vaccine at 9 to 12 months of age. Because currently licensed vaccines have lower than desired efficacy in very young infants, there has been research toward developing an efficacious vaccine that can be safely administered earlier in infancy. Additionally, there is a potential future need for an improved measles vaccine for future immunization schedules that will evolve to emphasize administration of vaccines at earlier ages in infancy and will use multiple combinations of vaccines.

Most recent research to develop improved measles vaccines has concentrated on the selection of more potent measles vaccine strains, or the development of high-titer vaccine formulations, that might effectively immunize a higher percentage of vaccinees and might be given at 6 months of age or less. However, studies in some parts of the world showed that high-titer vaccines might be associated with an increase in childhood mortality during a period of up to 2 years following immunization at 6 months of age. Although the reasons for this are not known, it might be related to the immunosuppression that results from natural measles and also might occur with high-titer vaccines. Consequently, in 1992, the WHO recommended that high-titer measles vaccines not be used.

Unfortunately, measles is a difficult virus to study because there are no satisfactory animal models. Within the last 2 years, both basic and applied measles vaccine studies have been accelerated by complementary WHO and National Institutes of Health funding for the development of a reliable measles monkey model. Also, much progress has been made in applying basic molecular virology approaches to define the genetic, molecular, and antigenic characteristics of measles. After elucidation of the molecular structure of this virus, the major emphasis of researchers has been to express antigens (particularly antigenic sites on H, F, M, and N proteins) in a form suitable for use as a vaccine. A Request for Applications issued by NIAID in late 1992 stimulated measles research and resulted in the development of a number of potentially new measles vaccine candidates including ISCOMs (immunostimulatory complexes), nucleic acid vaccines, pox-vectored vaccines, viral subunit immunogens, and BCG (Bacille Calmette-Guérin)-vectored vaccines. Additionally, this research program further advanced the development of the new primate model systems. These systems have been used to directly compare the immunogenicity of potential new vaccine candidates in nonimmune monkeys and in monkeys passively given measles antibody to mimic maternal antibody. These primates have also been given standard vaccine, high-titer vaccine, and older killed vaccine (both frozen old stocks and re-created 1960s era products) in an attempt to use modern immunological tools to determine what caused the vaccine-related accidents with inactivated vaccine in the 1960s, and with live high-titer vaccine in the 1990s. To date, although data on nucleic acid vaccines in primates are incomplete, it appears that ISCOMs and the nucleic acid vaccine have the greatest potential for inducing a protective immune response in the presence of maternal antibodies.

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Mumps

Mumps vaccine was licensed in the United States in 1967, and the number of cases has dropped over 99 percent to 751 in 1996. This drastic drop in cases occurred because of an increasingly inclusive vaccination policy at a State and Federal level. The recent introduction of a second immunization of measles using measles, mumps, and rubella (MMR) vaccine has accelerated reduction of mumps cases.

As opposed to polio and measles, elimination of mumps has not been an important global health goal. However, a recent study indicates that elimination of mumps might not only eliminate the acute mumps illness, but might also eradicate endocardial fibroelastosis. The study screened for the presence of genome material of various viruses in autopsy tissue from 29 pediatric patients with endocardial fibroelastosis. This study included tissue samples from 1955 to 1992, and over 70 percent of the heart tissue contained genetic material from the mumps virus. Only 1 of 65 matched controls contained any viral material and that was to an enterovirus. Endocardial fibroelastosis was once common, occurring in 1 of 5,000 births, but the cases have declined sharply. Interestingly, almost all the tissue samples before 1980 contained mumps viral material, whereas none after 1980 contained mumps.

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Rubella

Worldwide, rubella remains a common benign febrile disease of childhood. The most serious effects of rubella—spontaneous abortions, miscarriages, stillbirths, and congenital rubella syndrome (CRS)—follow infection during early pregnancy. The currently licensed vaccine is highly effective, and its combined use with measles and mumps vaccines in childhood immunization programs has drastically reduced the cases of rubella in the United States. From 1969 to 1989, the number of reported annual rubella cases dropped 99.6 percent. Although there was a slight reemergence of rubella cases between 1989 and 1992, from 1992 to 1996 an average of only 183 rubella cases occurred annually. Most recently, from 1994 to 1996, 18 States reported no rubella cases, and the majority of cases were clustered in six outbreaks scattered across the United States. It is estimated that the average cost of a single case of CRS is more than $500,000. Sixty-seven cases of CRS were reported in the United States in 1970, the year the vaccine was licensed, and except for the reemergence of CRS in the early 1990s (33 cases in 1991), CRS cases have steadily declined, with only 12 cases reported from 1994 to 1996. Encouraged by this success, indigenous rubella and CRS have been targeted for elimination in the United States by the year 2000.

It can be generally concluded that in the developing world, natural rubella infection occurred early in life and almost universally. In such a situation, unless the rubella epidemiology changed, there was no pressing need to immunize against rubella. However, recently it has came to the attention of WHO officials that many countries have, on their own, purchased MMR for their measles campaigns, and thus have already started to alter the natural circulation of rubella. Once this interference has occurred, and "natural immunization" with rubella is not universal, rubella immunization programs must be aggressively continued. Consequently, rubella control and eradication have again been thrown into the public health spotlight.

The epidemiology of rubella in the United States has changed from the 1980s such that since 1994, 84 percent of the cases occur in patients older than 15. Apparently, most cases occur among unvaccinated adults. Ninety-three percent of cases were indigenous to the United States; many imported cases came from countries that do not routinely provide rubella immunization (e.g., Mexico). From 1991 to 1996, the percentage of cases among Hispanics increased from 19 percent to 68 percent. Thus, future immunization programs will focus more efforts on adolescents

and adults, and on selected ethnic groups that have lower rates of immunization and close contact with people coming from countries without comprehensive rubella immunization. Obviously, interruption of U.S. rubella cases would benefit from improved global immunization programs.

Although the total number of cases of rubella is low and the number of cases of CRS is limited, the recent reemergence of natural rubella led to a campaign to increase vaccination coverage in all U.S. age groups. Consequently, many adult women were immunized against rubella, and a longstanding concern was again raised about possible vaccine-associated arthritic complications in these women. Early reports of naturally occurring rubella epidemics noted an increased incidence of arthropathy, predominantly in adult women. Like natural rubella, there are reports that the vaccine causes transient joint symptoms in a significant proportion of women vaccinees. Joint complaints have been reported in up to 25 percent of previously seronegative vaccinees; these symptoms may last from 1 day to 3 weeks after immunization. Investigators in Canada had reported preliminary data indicating that a small percentage of adult female vaccinees develop a more severe and persistent arthropathy. One suggestion was that these complications might increase with the age of the vaccinee and/or the presence of low—or incomplete—rubella immunity. The causal relationship of rubella vaccination to the acute type of arthritis was highlighted in a recent Institute of Medicine report on vaccine safety, but its relationship to chronic arthritis remains unclear. In the past few years, two large studies of immunized populations suggest that the long-term types of arthritic complications are not commonly associated with rubella immunization. More basic research studies have shown that for rubella virus to replicate, it must bind to host cellular proteins. These proteins are under investigation, as is their role as autoantigens and their potential to contribute to arthropathy.

Basic research on rubella is now proceeding at a reduced level of funding, and the National Institute of Allergy and Infectious Diseases supports only one project dealing with rubella. Current research is focused on identifying and characterizing virus gene products required for generating long-lasting immunity, as well as those associated with the expression of adverse effects. If one hopes to improve the current vaccine so that it will protect women of childbearing age without causing undesirable side effects, the basic research base will have to be expanded.

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Meningococcal Diseases

Background

Neisseria meningitidis (NM) is the leading cause of bacterial meningitis and continues to be a major public health problem, not only in the United States but also worldwide. Although the disease has a more severe impact on children and young adults, all age groups are susceptible to infections. The disease is transmitted from person to person by close contact. In the United States there are an estimated 3,000 cases per year involving meningococcal serogroups B, C, and recently Y; however, in other parts of the world, the number of cases is much higher. For example, in sub-Saharan Africa, during the 1996 epidemics caused by serogroup A, more than 200,000 cases were reported, with 20,000 deaths. A significant proportion of the children who survive infections caused by *N. meningitidis* have permanent side effects such as deafness. The emergence of new strains of meningococci and penicillin-resistant meningococci in the United States has further complicated the picture and caused serious public health concerns.

A major gap in the understanding of the pathogenesis of meningococcal disease (MD) is the relationship between carriage of meningococci and invasive MD. Most meningococci possess a polysaccharide capsule, which forms the basis of classification into serogroups. The presence of the capsule helps the organism resist phagocytosis. Recent studies show that the capsule not only alters the adherence of the organisms to leukocytes but also alters the interaction with lysosomes within the cells. An additional virulence mechanism is the ability of meningococci to escape protective immunity by switching capsules. The organisms also carry pili that facilitate adherence to host cells and possess a large number of outer membrane proteins (OMP) including Opc and Opa, which appear to mediate invasion of epithelial cells. Further, some of these proteins (e.g., the pili and Opas) show considerable antigenic variation. In addition, the organisms are capable of secreting proteins (e.g., FrpA and FrpC) with potential toxicity; however, the role of these toxins in MD has not been established. Meningococci can secrete IgA protease, which can cleave human IgA; however, its role in pathogenesis is still not clear. Another important viru-

lence factor is endotoxin. Unlike the endotoxin of Enterobacteria, this molecule contains short sugar chains and hence is termed lipooligosaccharide (LOS). Studies indicate that LOS is important for colonization in the nasopharynx. Additionally, the release of meningococcal LOS contributes to the hypotension and shock associated with fulminant meningococcemia. Also, LOS and other meningococcal components can induce a variety of cytokines and other mediators of the immune response that have a significant impact on the course of the infections.

Although meningococci are carried asymptomatically in the nasopharynx of 5 to 10 percent of normal individuals during nonendemic periods, it is still not clear why some individuals become susceptible to invasive MD. It is known that individuals with complement deficiencies and malnourished, immunosuppressed, or asplenic patients are particularly at high risk. The results of a recent study designed to address the issue of genetic predisposition to MD suggest that there is a genetic inheritance pattern among families with respect to the amount of cytokines produced. These results also suggest that the type of cytokines produced may be associated with the risk of fatal disease. More recent studies have demonstrated the presence of decreased plasma levels of coagulation factors and increased expression of cellular adhesion molecules in meningococcal patients and shown that interleukin-12, tumor necrosis factor, and interferon-gamma may contribute to natural immunity.

Current Status in Research and Development

Considerable efforts have been focused on vaccine development. The currently licensed vaccines based on purified capsular polysaccharides (PS) from four major serogroups (A, C, W135, and Y) are moderately immunogenic, but the immune response, in general, is of short duration and cannot be boosted upon re-immunization, and the polysaccharide vaccines do not elicit an immune response in children less than 2 years of age. Interestingly, group A capsular polysaccharide vaccine is moderately immunogenic in this age group; the underlying mechanisms of this unique response are not clear. A current attractive strategy in vaccine development is to use polysaccharide-protein conjugate vaccines to enhance the immunogenicity and to induce memory.

Although major advances have been made in the development of vaccines for group A and C strains of meningococci, there are no licensed vaccines for group B meningococcal infections in the United States and the development of vaccines against group B strains remains problematic. Unlike the other meningococcal capsular PSs, the group B PS is poorly immunogenic in both infants and adults. Recent studies using x-ray crystallography suggest that the poor immunogenicity may be due, at least in part, to the fact that the conformational epitope of group B PS that is capable of inducing an immune response may not be stable under different physiological and pathological conditions. Because group B strains continue to be a major cause of meningococcal disease in the United States and several other countries, the development of an effective group B capsular PS vaccine would represent a major advance in the prevention of meningococcal disease. However, there are important concerns that such a vaccine might induce immunopathology such as the formation of cross-reactive autoantibodies to specific oligosaccharides also found on mammalian cells. For example, anti-group B PS antibodies cross-react with the neural cellular adhesion molecule, a membrane glycoprotein involved in cell-cell adhesion. Therefore, it is possible that a PS-based vaccine may induce immunopathological side effects.

Such concerns have prompted the pursuit of alternative strategies for group B vaccine development using mainly meningococcal outer membrane proteins but also targeting lactoferrin and transferrin-binding proteins. Studies indicate that protection can be induced by OMPs. For instance, it has been shown in an infant rat model that antibodies to PorA proteins are protective against meningococcal infections. Protein-based vaccines have been used in clinical trials in Cuba, Brazil, Chile, and Norway with efficacies ranging from 50 to 80 percent. Unfortunately, these vaccines induced no protection in children and the immune response was of short duration. Recent vaccine approaches include (1) a multivalent OMP vesicle vaccine in which vaccine strains were constructed by recombinant DNA techniques to express three different PorA proteins that is currently undergoing clinical trials; (2) an A/B (chemically modified group B PS)/C combination vaccine; and (3) and an anti-idiotype group B vaccine.

Recent Accomplishments and Developments

Risk for meningococcal disease may be inherited

Studies show that although meningococci can be carried in the nasopharynx of healthy individuals, the organism can sometimes escape and invade the bloodstream and subsequently spread throughout the body. Although it is clear that some individuals are more vulnerable than others to meningococcal disease, the underlying events that permit the organisms to become invasive in a susceptible host still remain elusive. In an attempt to clarify some of these issues, a team of researchers, led by Dr. Westendrop of the University of Leiden, studied immune responses of a group of related individuals (parents, siblings, and children). The results of these studies suggest that there is an inheritance pattern with respect to the amount of cytokines produced. In addition, there was an interesting correlation between the type of cytokine produced and the risk of fatal disease.

Generation of immunological memory to meningococcal polysaccharide in children by using conjugate vaccines

Major efforts have been made to develop meningococcal-protein conjugate vaccines because of previous observations that the purified polysaccharide was not immunogenic in children and did not induce a memory response. In a recent study using a conjugate in The Gambia, it was noted that children who had previously received the vaccine at 6 months of age gave significantly higher antibodies to group C polysaccharide when revaccinated at 24 months of age.

Neisserial porins can enhance immune responses

The development of vaccines has been useful in the prevention of a variety of infectious diseases. In the process it is often desirable to enhance the immune responses of vaccine candidates by using adjuvants. Previous studies conducted by Dr. Wetzler have examined the ability of Neisserial outer membrane proteins (mainly porins) to augment the immune response to a variety of anti-

gens. These studies have shown that porins can increase the antibody response to peptides, polysaccharides, and glycolipids. The most recent investigations have focused on the molecular mechanisms by which porins enhance the cellular and humoral immune responses. Specifically, these studies have examined the expression of critical cellular adhesion molecules on the surface of immunocompetent lymphocytes as well as an analysis of the intracellular signal transduction events involved.

Meningococcal endotoxin is a critical factor in causing disease

In spite of effective antibiotics and partially effective vaccines, meningococcal disease remains a major public health concern. Meningococci can overcome the normal defense mechanisms of a susceptible host. Studies show that a critical virulence factor that is important for colonization of the host cells is lipooligosaccharide or endotoxin. In addition, the severity of the disease has been correlated with the release of LOS into the bloodstream. As such, it has been used as a component for meningococal vaccines in clinical trials. Although it is clear that LOS is important in the events leading to bacteremia and meningitis, the precise role in the events leading to dissemination and invasion is still not well defined. Dr. Stephens' work focuses on elucidation of the structure of LOS by using molecular and biochemical techniques and on the interaction of LOS with host cells. These studies will add to the understanding of the role of LOS in meningococcal infections.

Future Steps/Challenges

The development of a new and improved vaccine, in the context of "optimal" adjuvant/delivery system, that is safe and immunogenic in children would have a tremendous impact in decreasing the incidence of the disease. Studies using a number of adjuvants including monophosphoryl lipid A and Quil A to enhance the immune response to meningococcal vaccines represent a significant advance. Also, basic research studies should be encouraged to analyze the biological, structural, and molecular aspects of potential virulence factors and to identify novel bacterial components that may serve as potential vaccine targets. It is hoped that once an adequate meningococcal vaccine is developed, meningococcal vaccination would be integrated into the WHO Expanded Programme of Immunization.

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Moraxella catarrhalis

Moraxella (Branhamella) catarrhalis, once thought to be a harmless commensal organism, has become recognized over the past decade as an important human pathogen. Today, *M. catarrhalis* is the third most common cause of bacterial otitis media in children, after *Streptococcus pneumoniae* and nontypeable *Haemophilus influenzae*. Otitis media is a major cause of morbidity in the pediatric population in developed countries and is the most frequent diagnosis made by health care providers in this age group in the acute health care setting. It is estimated that 3.5 million episodes of otitis media per year are caused by *B. catarrhalis*. An effective otitis media vaccine will, most likely, need to provide immunity to all three organisms. *M. catarrhalis* is also a frequent cause of sinusitis in this age group. In adults, this organism is an important cause of lower respiratory tract infections, particularly in the setting of chronic obstructive pulmonary disease (COPD) where it has become the third most common bacterial agent responsible for acute exacerbations of COPD. The organism also plays a significant role in other lower respiratory tract infections in adults, including pneumonia and laryngotracheobronchitis and is infrequently the cause of septicemia, meningitis, and endocarditis in immunocompromised adults.

Work has progressed rapidly during the past several years to identify two major outer membrane proteins, OMP CD and OMP E, associated with *B. catarrhalis*. Both proteins are considered potential vaccine candidates to prevent infections caused by this bacterium. So far, the genes have been cloned and the character-

istics of these proteins have been studied. These proteins are abundantly expressed on the bacterial surface and show a high degree of similarity from strain to strain. These two characteristics are important as potential vaccine antigens. Since a protein antigen is likely to be immunogenic in infants, this is likely to be an important consideration in preventing otitis media. Work is in progress to define the precise structure and epitopes of these proteins and to test rigorously whether antibodies to these proteins will protect against infection caused by *B. catarrhalis*.

Two other highly conserved OMPs also have been investigated as potential vaccine candidates. Both of these antigens, referred to as B1 and LBP, are iron-regulated proteins found on the surface of this Gram-negative pathogen in response to iron-limiting conditions in its environment. Several studies have been conducted demonstrating the importance of these surface proteins and their exposed epitopes in the pathogenesis of disease and for survival in the host.

Finally, efforts are under way to develop a serotyping system based on the iron-repressible OMP B2, which has a high degree of antigenic and sequence heterogeneity. Restriction fragment length polymorphism analysis indicates that the pattern of variable and constant areas in the B2 gene is a general pattern among all strains of *B. catarrhalis*. Developing such a serotyping system for strains of *B. catarrhalis* will be important to understand the epidemiology of infection to guide future vaccine studies with this organism.

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Mycoplasma pneumoniae

Background

In the United States, about 15 million respiratory infections are caused by *Mycoplasma pneumoniae* each year. *M. pneumoniae* is the leading cause of pneumonia in older children and young adults but also affects adults and elderly individuals. This microorganism is responsible for 25 percent of all cases of pneumonia requiring hospitalization and 50 percent of all pneumonias in closed populations and is the second leading cause of tracheobronchitis in children. A related organism, *M. hominis*, causes pyelonephritis in adults and pulmonary disease in neonates.

Mycoplasmas are wall-less prokaryotes that are biosynthetically deficient in several respects. Therefore, they must rely on the microenvironment provided by the host to obtain essential metabolites (nucleotides, fatty acids, sterols, and amino acids) needed for growth. Mycoplasmas possess a circular double-stranded DNA chromosome ranging from 600 to 1,300 kilobases, with complex genetic recombination systems and large genome families. The organism has a tremendous capacity to generate antigenic and phase variations that may be important in disease pathogenesis and tissue tropism, but this characteristic poses a special challenge for vaccine development. The complete genome of *M. pneumoniae* has been sequenced, and it is anticipated that this will shift the focus of research from gene structure to gene function and significantly advance the understanding and knowledge of the physiological and genetic characteristics and may provide new leads for vaccine development.

Although mycoplasmas are responsible for a variety of important diseases in humans and various animal species, experimental vaccines have not affected the spread of infection, possibly the result of the organism's ability to develop antigenic changes at high frequency.

In animal models, an inactivated *M. pneumoniae* vaccine induced a humoral immune response but was unable to stimulate a cell-mediated immune response. An experimental *M. pulmonis* (the agent of murine mycoplasmosis) vaccine has been shown to induce protective antibodies, but since a direct correlation has been shown between a respiratory secretory IgA (sIgA) antibody response and resistance to *M. pneumoniae*, other approaches may be necessary. An oral adenovirus *M. pneumoniae* vaccine is being developed to evaluate the role of sIgA antibody in the development of immunity. These preliminary studies may pave the way for rapid development of safe and effective vaccines against *M. pneumoniae* infections.

Earlier challenge studies conducted in human volunteers demonstrated that an inactivated *M. pneumoniae* vaccine was moderately protective, but these studies have not been developed further. Although the development of an appropriate animal model continues to hold back *M. pneumoniae* vaccine development, studies done in chimpanzees indicate that animals immunized with a formalin-inactivated vaccine or an acellular extract developed milder disease and lower colonization rates with mycoplasma compared with unimmunized controls. Because only partial protection was observed in such experiments, more studies are needed to increase the level of protection expressed. Other studies suggest that the manner in which immunogens are delivered may be an important factor in the generation of an optimal immune response.

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Parainfluenza Virus

Four serotypes of human parainfluenza viruses (HPIV 1-4) are associated with respiratory illness. A variety of upper respiratory infections caused by HPIVs include otitis media, pharyngitis, conjunctivitis, and the common cold. These can occur alone or in combination with the following lower respiratory tract infections: croup, laryngitis, bronchiolitis, or bronchopneumonia. HPIV types 1-3 cause croup and laryngitis in infants and children. Although HPIV-1 and HPIV-2 generally cause disease in toddlers and preschoolers, HPIV-3 is unique among the parainfluenza viruses in its ability to infect young infants less than 6 months of age. The most common clinical syndromes caused by HPIV-3 are bronchopneumonia and bronchiolitis. HPIV-3 infections are second only to respiratory syncytial virus infections as a cause of serious respiratory tract disease in infants and children. HPIV-4 has been associated with mild upper respiratory tract disease in children and adults.

Antigenic subtypes are found within the HPIVs, however; temporal and progressive variability has been associated with both HPIV-1 and HPIV-3. There are two antigenic subgroups of HPIV-4, A and B, based on antigenic differences detected by hemadsorption-inhibition and monoclonal antibody reactivity. The antigenic variability may affect the efficacy of HPIV-1 and HPIV-3 vaccines being evaluated.

One approach to vaccine development has been purification of major outer membrane proteins (fusion [F] and hemagglutinin-neuraminidase [HN] glycoproteins) for use as immunogens in subunit vaccine candidates. A current effort involves using ion-exchange chromatography to purify HN and F detergent-solubilized proteins from PIV 1, 2, and 3. When combined with the adjuvant, aluminum phosphate, this trivalent formulation evoked serum PIV 1, 2, and 3 specific neutralizing antibody titers in mice.

Another approach involves the development of live-attenuated parainfluenza (PIV) virus candidates, including a bovine PIV-3 and cold-passaged (cp) HPIV-3 vaccines. Clinical evaluation of cp-18, a cold-adapted, temperature-sensitive candidate vaccine derived by cold-passage of the JS strain of HPIV-3, demonstrated that it was attenuated in adults but was not satisfactorily attenuated in children, causing rhinorrhea and wheezing in two of four PIV-3 seronegative children. Because cp-45 was more attenuated than cp-18 in nonhuman primate studies, the safety, infectivity, and immunogenicity of cp-45 were evaluated in children 6 months to 10 years old. In this age group, the vaccine candidate cp-45 was well tolerated when given intranasally to PIV-3 seropositive and seronegative children. A dose-response study in seronegative children in this study demonstrated infectivity and immunogenicity at doses 10^2 to 10^5 pfu / ml. Further evaluation of cp-45 will be continued through a collaborative research agreement between NIAID and Wyeth-Lederle Pediatric Vaccines.

A bovine PIV-3 vaccine was chosen as a candidate live-virus vaccine because it is antigenically related to HPIV-3, as shown by sequence analyses of BPIV and HPIV HN and F glycoproteins and cross-neutralization studies. The first phase I trial demonstrated that BPIV-3 was safe, infectious, immunogenic, and phenotypically stable when administered to 6- to 36-month-old PIV-3 seronegative infants and children. The second study evaluated the BPIV-3 vaccine in two age groups (i.e., 2- to 6-month-old infants and 6- to 36-month-old infants and children). The vaccine was well tolerated in both age groups and infected 92 percent of infants younger than 6 months and 89 percent of infants and children older than 6 months. Serum hemagglutination-inhibition antibody responses to HPIV-3 and to BPIV-3, respectively, were detected in 42 and 67 percent of the younger infants, compared with 70 and 85 percent of the older group. Additional studies are needed to determine whether two or more doses will enhance the immunogenicity of the BPIV-3 vaccine in young infants.

No licensed parainfluenza vaccines are available. Although several approaches have been examined, the work that is currently further along involves live-virus vaccine candidates. The effort to produce a live-attenuated HPIV-3 vaccine will be assisted by the recent report describing the generation of infectious HPIV-3 from a full-length clone of the HPIV-3 genome, an important research advance supported by NIAID. Analysis of mutations within the infectious clone will allow identification of mutations that attenuate the virus and could be used to develop new vaccine strains. In addition, this technology provides the ability to produce infectious HPIV-3 engineered to contain specific alterations within the HPIV-3 genes to produce a highly attenuated virus that would not readily be able to revert.

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Pertussis

In the 1930s, pertussis (whooping cough) afflicted more than 190,000 children a year in the United States and killed as many as 9,200. Despite introduction of the "whole-cell" pertussis vaccine over 45 years ago, pertussis continues to occur among well-immunized populations in the United States. Of the 2,500 to 6,500 cases reported annually during the past 10 years, 67 percent occurred in children less than 5 years of age and 45 percent in infants. Of these children, 63 percent were not appropriately immunized for age and 34 percent had no pertussis immunizations at all. The morbidity of reported cases of pertussis remains high, particularly during infancy. Sixty-five percent of infants are hospitalized, 17 percent develop pneumonia, 2.5 percent develop seizures, 1 percent develop encephalopathy, and 0.5 percent die. The World Health Organization estimates that 50 million cases of disease occur each year worldwide, with an associated death toll of 350,000, mostly among unvaccinated populations. In India alone, 185,000 lethal cases of whooping cough occur each year, with commensurate levels in other developing countries.

Pertussis still threatens the United States from within; adults in the United States continue to harbor *Bordetella pertussis* and will likely infect infants if the infants are not vaccinated. Because a few well-publicized events could rapidly and irrationally destroy confidence in the current U.S. vaccination program, it is important to expeditiously develop acceptable alternative vaccines before a crisis occurs in this country. The initial phase of this work has been completed successfully, and three acellular vaccines were licensed in the United States within the past year for use in infants as part of the primary and booster series of immunizations. Two additional acellular vaccines are expected to be licensed in 1997. A multicenter vaccine trial in adolescents and adults has also begun to determine the epidemiology and efficacy of acellular pertussis vaccines in this population. It is hoped that the routine use of acellular pertussis vaccines in older age groups will help eliminate the primary reservoir of this organism.

The results from Trial II of the Swedish pertussis efficacy study were recently announced in Sweden. This study followed an earlier phase III pertussis trial that ended in 1995 and used a U.S. schedule of 2, 4, and 6 months of age. This latter study showed 85 percent efficacy for a Pasteur Merieux Connaught five-component acellular vaccine, but only 54 percent for a SmithKline Beecham (SKB) two-component vaccine. Trial II was designed to examine the relative efficacy of three acellular vaccines (i.e., a Connaught hybrid five-component vaccine, a Chiron Vaccine [CV] three-component vaccine containing a recombinant form of pertussis toxin [PT], and a SKB two-component vaccine) compared to a whole cell vaccine produced by Evans Medical, Inc., Great Britain. Approximately 72,000 infants were enrolled to receive vaccine at 3, 5, and 12 months of age, while another 10,000 infants received vaccine following a 2-, 4-, and 6-month schedule. The data indicate that the three- and five-component acellular vaccines and the whole cell product were highly effective in preventing whooping cough, with the latter two providing the best protection against mild forms of the disease. These findings suggest an important role for fimbriae, especially with regard to protecting against mild disease. This is important since so-called mild disease, less than 7 days of coughing, is thought to play a key role in transmission of the illness. In serious cases, children are usually isolated or hospitalized, but children with mild cases often continue their regular routines. Indeed, many mild cases may not even be recognized as pertussis. All acellular vaccines were significantly less reactogenic than the whole cell vaccine for several serious events categories such as temperature greater than 40.5 degrees centigrade and seizures. Following three doses, the 3-, 5-, and 12-month schedule was more effective in providing protection than the 2-, 4-, and 6-month schedule. This finding was expected since the third dose at 12 months is considered a booster dose, comparable to the fourth dose provided in the United States at 15 to 18 months.

Followup studies 3 years postimmunization continue to show sustained safety and efficacy for the three- and five-component acellular pertussis vaccines tested in Italy and Sweden. During the next several years, additional cell-mediated immunity, household contact, and relative risk studies will be conducted in Italy for the three-component SKB and Biocine vaccines. In Sweden, investigators will continue to analyze data to look for possible serological correlates of protection and information on the transmission of disease. In addition, followup studies to determine the presence of long-term sequelae will continue in children who experienced severe adverse events such as hypotonic-hyporesponsive episodes and seizures. Using hospital registries and other linked data sets, efforts will continue to examine whether there is any correlation between vaccination status and the prevalence of such maladies as juvenile-onset diabetes, asthma, sudden infant death syndrome, idiopathic thrombocytopenic purpura, and meningitis. The results so far indicate no difference in the incidence rates of diabetes among children with high and low exposure to pertussis vaccines.

A prospective, randomized, double-blinded, controlled, multisite trial has begun at five NIAID-sponsored Vaccine and Treatment Evaluation Units (VTEU) and three other contracted clinical sites. The purpose of the study is to evaluate the clinical spectrum and burden of pertussis infection in adolescents and adults and to determine the protective efficacy of a three-component acellular pertussis vaccine produced by SmithKline Beecham in approximately 2,000 healthy adolescents and adults aged 15 to 65 years. Subjects are being recruited primarily from schools and places of employment, including hospitals and medical centers. Participants are randomized to receive a single dose of either the pertussis vaccine or a control vaccine (SKB hepatitis A vaccine). In addition to protective efficacy, the safety of both vaccines, the immunogenicity of the acellular pertussis vaccine, and the pertussis-specific cell-mediated immune responses will be assessed. Epidemiological studies of *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* will also be conducted.

A multicenter booster study was recently completed in NIAID's VTEUs. Children, 4 to 6 years of age, who had been primed and boosted previously with one of several available acellular pertussis vaccines or whole-cell vaccine, received a fifth dose booster using the same vaccine. Regardless of which diphtheria-tetanus-pertussis (DTP) vaccine was given, common reactions, particularly local reactions, increased after the fifth booster dose compared with the primary series. Nevertheless, acellular vaccines generally produced a lower frequency of reactions than whole-cell vaccine and a higher serum antibody response. The best antibody responses were seen in children primed with whole-cell vaccine and boosted with an acellular product.

Pertussis-specific cell-mediated immunity was evaluated in infants after vaccination with a tricomponent acellular pertussis

vaccine produced by SmithKline Beecham. Infants were investigated following a three-dose primary vaccination schedule from the third month of life to the sixth month as well as before and after a booster at 15 to 24 months. This is the first report of specific cell-mediated immune responses to pertussis-related antigens in infants below the age of 12 months. The data show that the vaccine induces T cell responses specific for the vaccine components (i.e., detoxified pertussis toxin, filamentous hemagglutinin, and pertactin), which increase progressively over the course of the vaccination schedule. In contrast to declining antibody titers, cell-mediated immune responses were stable over the postprimary to prebooster period. Vaccination results in a progressive increase in the number of CD4-positive T cells after stimulation with pertussis antigens. Measurements of cytokine secretion profiles demonstrated a preferential induction of interleukin-2- and gamma interferon-producing T-helper 1 cells and only low production of interleukin-10, suggesting a low Th2-type cytokine profile. The observed persistence of the specific cell-mediated immunity may have a bearing on the protective mechanisms induced by pertussis vaccination. There is increasing evidence supporting a role for a specific cell-mediated immune response in the complete elimination of *B. pertussis* and subsequent protection against the disease. This study documents that inoculating infants with an acellular pertussis vaccine induces a strong and persisting T cell response to all vaccine antigens.

As indicated earlier, recent clinical studies have demonstrated the efficacy of three doses of acellular pertussis vaccines in preventing whooping cough, when administered in infancy, but little data are available on the duration of protection and the need and timing of booster doses. A large randomized clinical trial in Italy evaluated two vaccines, given as DTaP—one a three-component vaccine manufactured by SKB and the other a three-component vaccine manufactured by Chiron Vaccines. Both vaccines are licensed for use in selected European countries. Each vaccine was found to be 84 percent efficacious when children through the average age of 24 months were observed. To address the need for possible additional doses of vaccine and to continue to evaluate the persistence of protection provided by the primary immunization, a long-term prospective population-based observational study was conducted. The results of this continued surveillance led to the following conclusions: Three doses of each DTaP vaccine conferred a persisting high protection against whooping cough during the first 4 years of life, including periods of high incidence of pertussis and in the absence of additional vaccine administration. However, during the period between 19 and 27 months after primary immunization, a statistically significant difference in protection was detected between the two acellular vaccines (i.e., 77.7 percent for SKB vaccine and 88.8 percent for CV). Nevertheless, by combining observations from the initial and additional followup periods, each vaccine still showed, overall, a high clinical efficacy through the cumulative observational period (i.e., 80 percent for SKB and 85 percent for CV).

Work is under way to develop a *Salmonella typhi*-based live-vector DTP vaccine that can be administered by mucosal (oral or intranasal) immunization with a single dose. Investigators have genetically engineered an attenuated strain of *S. typhi* (the bacterium that causes typhoid fever) that serves as a live oral vaccine against typhoid fever. In clinical trials carried out so far, one particular strain, CVD 908-*htr*A, has proven to be well tolerated and impressively immunogenic when administered as a single oral dose in humans. A basic strategy is to further engineer CVD 908-*htr*A by introducing genes that express protective antigens of *Corynebacterium diphtheriae*, *Clostridium tetani*, and *Bordetella pertussis*, the pathogenic microorganisms that cause diphtheria, tetanus, and pertussis, respectively. The *S. typhi* live vector would then express these foreign antigens and deliver them to the human immune system, resulting in a protective immune response. With this attenuated *S. typhi*-based live vector, a fusion protein was successfully expressed consisting of a truncated S1 subunit from pertussis toxin fused to fragment C, a nontoxic antigen obtained from tetanus toxin. Following intranasal immunization of mice, this construct elicited serum antitoxin that was able to neutralize pertussis toxin. Challenge studies in mice are about to begin. Other pertussis antigens such as filamentous hemagglutinin and pertactin have also been expressed in *S. typhi*, and preclinical studies in mice are under way as well.

The whooping cough organism, *B. pertussis*, produces a protein toxin that enters tissues of infected children and causes tissue damage. The toxin is an enzyme that attacks and destroys the metabolic regulatory system of respiratory tissues. Loss of metabolic control causes respiratory injury and can lead to tissue death. Research in one of NIAID's grant-supported programs has provided a detailed understanding of the chemical step used by whooping cough toxin to cause its tissue damage. This information is now being used as a blueprint to develop transition state inhibitors directed against the whooping cough toxin. Although it is early in the development of this program, this new approach has great promise because agents against the toxin will prevent tissue damage in children who are already infected with *B. pertussis*. This approach departs from conventional antibiotic treatment because it cannot cause the formation of antibiotic resistance. It is expected that this work will provide detailed chemical knowledge of how whooping cough toxin works, and it represents a major step toward the design of novel therapies against this important childhood disease. New classes of antibiotics that prevent tissue damage and do not cause the development of antibiotic resistance could play an important role in the armamentarium against infectious diseases in general.

Investigators in Holland have begun to define the population structure of extant *B. pertussis* strains around the world, focusing on Dutch strains initially. Not surprisingly, they have found that the introduction of a vaccine into a human population causes skewing of the extant *B. pertussis* clones toward those not represented in the vaccine. Outbreak years in Holland are characterized by the spread of a few clones, whereas endemic activity reflects the presence of many (about 30 to 40) clones. The interest in these studies is in their potential to illuminate the mechanism of recent countrywide epidemics in Holland and other European countries, and in particular, to reveal whether the acellular pertussis vaccines may be selecting for the emergence of previously rare clones. The latter might carry polymorphisms ("escape mutations") in genes encoding the acellular vaccine antigens (e.g., pertactin, filamentous hemagglutinin, pertussis toxin). This has great potential significance for future vaccine strategies.

The three-dimensional structures of several pertussis virulence factors / antigens have now been revealed with x-ray crystallogra-

phy and high-resolution nuclear magnetic resonance. Pertactin, pertussis toxin, and filamentous hemagglutinin all reveal novel tertiary structural elements. The structures may help illuminate function as well as predict immunodominant regions, which will contribute significantly to the development of new types of effective vaccines. A three-dimensional structure of pertussis toxin could also provide a basis for designing cytotoxic agents coupled to cancer cell-targeting antibodies as well as contributing to our improved understanding of basic receptor biology and oncogenesis.

B. pertussis has been shown to inhibit antigen-dependent T cell proliferative responses. Human monocytes and macrophages bind *B. pertussis* through multiple specific receptor-ligand interactions; however, the effect of these interactions on monocyte and macrophage function is not well understood. In an *in vitro* system, *B. pertussis* infection of human monocytes significantly impaired T cell proliferation to exogenous antigen at multiplicities of infection as low as 1.0. *B. pertussis* isogenic mutant strains deficient in filamentous hemagglutinin or adenylate cyclase toxin were incapable of proliferation inhibition, suggesting that these virulence-associated factors are essential for this activity. *B. pertussis*-induced monocyte death alone did not explain these results, nor did differences in intracellular survival. In addition, *B. pertussis* infection did not significantly alter monocyte phagocytosis of complement-opsonized latex particles, indicating that *B. pertussis* infection does not globally impair monocyte functions in this system. These results suggest that *B. pertussis* may be capable of subverting cellular immune defenses in an infected host. Thus, *B. pertussis* may serve as an extremely useful model for understanding how respiratory (and other) bacterial pathogens modify the capabilities of the host immune system, to persist longer and facilitate spread to a new susceptible host.

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Pseudomonas aeruginosa

Background

Pseudomonas aeruginosa is an opportunistic organism as well as a pathogen for patients with cystic fibrosis (CF), a disease that usually presents itself in early childhood. Significant advances have been made in the management of CF patients through diet and physiotherapy and by treatment with human deoxyribonuclease I (rhDNase) to relieve airway obstruction. Now, many CF patients survive to adulthood. Although there has been considerable progress in the use of gene therapy to correct the basic genetic defect of CF at the molecular level, there is no evidence that gene therapy alters the course of *Pseudomonas* infection in this population so that preventive approaches, such as the development of safe and effective vaccines, are needed. Efforts to control these infections with antibiotics and better pulmonary therapy have done little to reduce the high mortality associated with *P. aeruginosa* pneumonia; however, immunotherapeutic interventions with active vaccination or passive therapy may have a significant impact on the development of sepsis and on survival. Indeed, the results of studies using passive immunotherapy with immune globulin enriched with anti-*P. aeruginosa* lipopolysaccharide antibodies indicate that this approach is effective.

While *P. aeruginosa* is a special problem for CF patients, it also contributes to the high mortality rates (>50 percent) in patients with emphysema, cancer, AIDS, and serious burns. The reason for the extraordinary pathogenicity of *P. aeruginosa* in these patients is not clear. However, it is possible that a variety of virulence factors produced by *P. aeruginosa* may account for the high mortality rates. Such virulence factors may play a role in colonization, tissue invasion, or the inhibition of a variety of immune responses. A major virulence factor produced by *P. aeruginosa* is an exopolysaccharide

or alginate. Alginate not only encapsulates the infecting bacteria, thereby protecting them from antibiotic treatment or from attack by host immune responses, but also enables the bacteria to adhere to epithelial cells of the lung and enhances the opportunity for further colonization and invasion. Other virulence factors associated with *Pseudomonas* infections include cell-associated structures, such as pili, as well as secreted products such as exotoxin A, exoenzyme S, hemolytic phospholipase, and proteases. Expression of these virulence factors is highly regulated, which probably accounts for the ability of *P. aeruginosa* to cause such a wide variety of infections in vastly different host environments.

Significant inflammatory changes are associated with *Pseudomonas* infections. It has been observed that CF patients have high levels of inflammatory cytokines (e.g., interleukin [IL]-8) in the lung environment relative to the levels in normal individuals. By contrast, the levels of cytokines, such as IL-10, that decrease inflammation are low in CF patients as compared to those in normal individuals. Recent molecular analysis of signal transduction mechanisms suggest that *P. aeruginosa* induces the epithelial cell production of IL-8 by activation of nuclear factor kappa B (NF-κB). Cells with CF mutations have significant endogenous levels of activated NF-κB. It is possible the mechanisms of signal transduction underlying the endogenous activation of NF-κB are different from the signals involved in the activation by *P. aeruginosa*. These inflammatory changes must be taken into account in the design of preventive procedures such as vaccines against *P. aeruginosa*.

Significant advances have been made in the development of vaccines against *P. aeruginosa*. Several surface proteins and polysaccharides have been demonstrated to be safe and immunogenic in small phase I/II studies, and have been shown to generate protective immunity in various animal model systems. For example, both high molecular weight polysaccharides and mucoid exopolysaccharide vaccine preparations have been tested in humans. Other vaccines based on outer membrane proteins or killed whole cell vaccine preparations have been found to be immunogenic in clinical trials. For instance, studies show that recombinant outer membrane protein I (oprI) is highly protective in experimental animals; and purified oprI was also found to be safe and immunogenic in clinical trials. In other studies, oral immunization with killed *P. aeruginosa* vaccine preparation protected naive animals against challenge with live bacteria. Despite these encouraging results, most studies done to date have demonstrated immunogenicity without protective efficacy.

Investigators have also pursued the use of recombinant OMPs as vaccines against *P. aeruginosa* infections. The results of experiments using a hybrid vaccine, containing protective epitopes of outer membrane proteins F and I, indicate that the vaccine was highly protective against *P. aeruginosa* infections in mice. In other studies, recombinant OMP I was also found to be safe and immunogenic in human volunteers. However, the use of OMPs as vaccines against *P. aeruginosa* infections requires further study.

For CF patients a vaccine should induce an immune response that would prevent mucosal colonization of *P. aeruginosa* and/or elicit a response against virulence factors associated with adherence. A better understanding of the molecular regulation of *P. aeruginosa* virulence factors and host response to infection should provide valuable information for vaccine design.

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Respiratory Syncytial Virus (RSV)

Respiratory syncytial virus is the single most important cause of severe lower respiratory tract infection in infants and young children. It is a common cause of winter outbreaks of acute respiratory disease and results in an estimated 90,000 hospitalizations and 4,500 deaths each year in the United States. The global annual infection and mortality figures for RSV are estimated to be 64 million and 160,000, respectively. RSV infects repeatedly and causes disease throughout life, including a wide array of respiratory symptoms from rhinitis and otitis media to pneumonia and bronchiolitis, with the latter two diseases having significant morbidity and mortality. RSV infects nearly all children by 2 years of age with reinfections during later childhood and adulthood that are generally associated with milder disease. RSV infections also occur in adults, with outbreaks reported among institutionalized elderly patients that were complicated with pneumonia. Severe RSV infections are a problem in immunocompromised patients of any age, especially transplant recipients. There is recent evidence of a link between RSV infection and the development of asthma.

The development of a RSV vaccine is a difficult but important priority. The most significant obstacle to developing a vaccine against RSV infections is the experience from clinical trials conducted in the 1960s to test a formalin-inactivated whole RSV vaccine in children. Recipients who were seronegative at the time of vaccination experienced lower respiratory tract disease of increased incidence and severity upon subsequent natural infection. To develop an effective vaccine, it is imperative to understand both the protective as well as the disease-enhancing immune responses to RSV. Research efforts have been focused on the individual components of these responses, including cell-mediated events as well as production of serum and secretory antibodies. Although much has been learned about these components, a safe and effective vaccine that induces protective immunity and does not cause enhanced disease is not yet available. An effective vac-

cine could be useful to reduce morbidity, reduce the frequency of hospitalization, and decrease the death rate. Vaccine candidates under development are evaluated in animal models first, followed by adults, immune children, older nonimmune children, younger nonimmune children, and susceptible infants.

Animal models that were developed to study RSV include the cotton rat, mouse, calf, lamb, and primates (bonnet monkey, African green monkey, chimpanzee) and have been useful in understanding and characterizing protective responses to RSV and vaccine candidates. In addition, data from these models are helping to elucidate the disease-enhancing reactions and are being used for improved strategies for vaccine development (i.e., histologic analyses and measurements of cell-mediated immune response following RSV infection). Recently a lot of formalin-inactivated RSV was made as a facsimile of the lot that caused enhanced disease in the 1960's clinical trials. This reagent has been available for investigations in animal models to further the understanding of the mechanism of disease enhancement.

There are two RSV strain subgroups, A and B. A successful vaccine would induce resistance to both subgroup A and B strains of RSV. Neutralizing antibodies are induced by F and G glycoproteins found on the surface of RSV. The major difference between RSV subgroups A and B is the G protein, which is responsible for attachment of RSV to a susceptible cell. The F surface protein is highly conserved among the RSV subgroups and functions to promote fusion of the virus and host cell membranes. In animal models of RSV infection, neutralizing antibodies against the F protein or G protein confer protection against homologous challenge, whereas antibodies against the F protein protect against heterologous challenge.

Purified F protein has been developed as a potential vaccine candidate. PFP-1 is a subunit vaccine that contains 5 to 10 percent of non-F proteins consisting mainly of G protein. PFP-2 contains less than 2 percent of non-F proteins due to purification by ion-exchange chromatography. Both PFP-1 and PFP-2 have been shown to be safe and immunogenic in studies with 12- to 48-month-old RSV-seropositive children. The efficacy of the vaccine could not be determined because of the low numbers of children in these studies. Subunit vaccines may be particularly useful in specific groups of high-risk children and adults. A recent study in children with cystic fibrosis demonstrated that PFP-2 vaccine induced a significant antibody response and a significant reduction in the number of lower respiratory tract illnesses. Another recent study demonstrated safety and immunogenicity of PFP-2 vaccine in ambulatory adults over age 60.

Maternal immunization using a purified F protein subunit vaccine is a strategy being evaluated to protect infants younger than 6 months of age from RSV disease. The rationale is based on reports of efficient transfer of specific maternal neutralizing antibodies to infants and demonstration of the prophylactic value of high-titer anti-RSV polyclonal antiserum administered to high-risk children (protection against lower respiratory tract RSV disease and hospitalization). The advantages of maternal immunization are that babies less than 6 months old are most at risk for RSV infection but are least responsive to vaccines; pregnant women respond well immunologically to vaccines; and placental transfer of maternal antibody occurs naturally during the third trimester.

A preliminary phase I feasibility study on the use of the purified F glycoprotein in postpartum women was completed in 1993 at Baylor College of Medicine. This initial study demonstrated that the vaccine was only minimally reactogenic and was highly immunogenic. A second study comparing the safety and immunogenicity of the PFP-2 vaccine with a licensed trivalent inactivated influenza virus vaccine was undertaken at Baylor College in 1994–1995. As with the pilot study, the RSV PFP-2 vaccine was only minimally reactogenic and highly immunogenic in postpartum women and women of childbearing age. Data suggest that this vaccine could potentially provide protective serum antibody to newborn infants.

A subunit approach has also been investigated using the G protein fragment of RSV-A Long strain. A novel recombinant vaccine candidate has been developed in which a polypeptide of the G protein (G2Na) of RSV Long strain was fused to BB, the albumin-binding region of streptococcal G protein, and resulted in the production of BBG2Na by prokaryotic expression in *Escherichia coli*, which induced protective immune responses in rodent models.

Another approach to vaccine development has been to construct live-attenuated RSV strains. Early attempts included cold passage, cold adaptation, chemical mutagenesis, temperature-sensitive selection, and combinations of these methods. Administration of live-attenuated virus preparations has not been associated with enhanced RSV disease upon subsequent natural reinfection. Problems that have impeded progress in this area are overattenuation, underattenuation, and concerns about genetic stability. As a result of recently developed technology, it is possible to introduce individual mutations into a cDNA clone of RSV and recover infectious virus, thus providing a mechanism to construct defined attenuated vaccine viruses with improved genetic stability. With this technology a specific mutation in a strain of cold-passaged RSV-A was recently analyzed in the cDNA-based recovery system and shown to be responsible for a temperature-sensitive phenotype. Scientists at the National Institutes of Health and in industry are working on live-attenuated vaccine candidates that have shown promising results in animal models.

Vaccinia or adenovirus recombinants expressing RSV F and/or G glycoproteins, as well as a bovine strain of RSV, have also been evaluated as vaccine candidates. Studies in chimpanzees have indicated that none of these are promising vaccine candidates.

The prospects for the future for RSV vaccines are encouraging. Ongoing studies are focused on furthering the understanding of protection and immunopotentiation of RSV disease, to provide the scientific basis required for the rational design of candidate RSV vaccines. Purified F protein subunit vaccines have been shown to be safe and immunogenic in seropositive children, postpartum women, women of childbearing age, and adults over 60. They have great potential use in adults and specific groups of high-risk children (cystic fibrosis) and for protecting infants via maternal immunization. Different adjuvants are currently being studied to augment immunogenicity. Live-attenuated vaccine candidates have also been shown to be safe and immunogenic. New methods in biotechnology are now available to provide tools for designing vaccines with defined mutations to achieve desired levels of attenuation that are genetically stable.

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Streptococcus pneumoniae

Pneumococci are the leading cause of death by infectious disease in the elderly and cause the majority of ear infections in young children. This organism is also an important cause of meningitis in young children and the elderly. Although ear infections in young children generally do not lead to meningitis or other serious pneumococcal diseases, they result in costly clinic visits for the children and much lost work by their parents. Because of its ability to infect the very young, the very old, and the immunodeficient, the pneumococcus has one of the largest public health and economic impacts of any infectious disease agent in the United States. Worldwide, the pneumococcus remains a leading bacterial pathogen in adults and the foremost cause of morbidity and mortality in infants and children in developing countries. Patients recovering from viral infections such as measles or influenza and those already afflicted with chronic diseases such as HIV infection constitute especially susceptible hosts in whom mortality from the coinfecting pneumococcus is high.

In recent years, pneumococci have developed resistance to most of the antibiotics now in use, a situation that leaves physicians without a satisfactory means of treating patients infected with these resistant strains. Even though over half of pneumococci remain susceptible to many antibiotics, since laboratory confirmation of antibiotic susceptibility patterns may take several days to complete, clinicians are often forced to initiate treatment with broad-spectrum antibiotics, a situation that only accelerates antimicrobial resistance. Therefore, preventing these infections with safe and effective vaccines will not only slow down the development of antibiotic resistance, but is an extremely cost-effective way to control pneumococcal disease. The present vaccine contains a mixture of 23 different polysaccharides. This vaccine is not immunogenic in young children and has only moderate efficacy in the elderly, a group at especially high risk of life-threatening pneumococcal infections. Therefore, a more effective vaccine for pneumococcal infection is a priority.

Much work has been done in developing pneumococcal conjugate vaccines as the next generation of promising vaccines against pneumococcal diseases. Widespread vaccination against virulent serotypes of *Streptococcus pneumoniae* could reduce infant mortality and protect against antibiotic resistance by inducing titers of mucosal antibodies sufficient to eliminate nasopharyngeal carriage. Currently, there are five active efficacy trials evaluating three different pneumococcal multivalent conjugate vaccines manufactured by two companies (i.e., Wyeth-Lederle Pediatric Vaccines and Merck Sharp Dohme). The end points for these trials vary considerably, from otitis media to invasive disease. During the next year, several additional efficacy trials are expected to begin at sites both within the United States and abroad. Once again, the emphasis will be on invasive disease as well as the impact of conjugate vaccines on colonization. Many of the conjugate vaccines in these new trials will contain as many as 11 serotypes (serotypes 1, 3, 4, 5, 6, 7, 9, 14, 18, 19, 23). During the next year, studies will also be conducted in several high-risk populations such as sickle cell anemic patients and the elderly to determine whether the conjugate vaccines offer any significant advantage over the conventional 23-valent capsular polysaccharide vaccine with regard to safety and immunogenicity.

The use of polysaccharide-protein conjugate vaccines, while overcoming many of the liabilities of polysaccharide vaccines, still involves a number of problems. First, new studies indicate that immunity induced by pneumococcal conjugate vaccines may be short-lived, especially in infants. Such a limitation would necessi-

tate repeat vaccinations through the first several years of life—an expensive procedure even in relatively wealthy nations, but an even greater and prohibitive expense for developing countries where cost factors play a major role in deciding whether a vaccine gets used or not. Secondly, regional variations in the predominance of infecting pneumococcal serotypes necessitate formulation of capsule-based vaccines that are appropriate to the local epidemiology. Such modifications are not only technologically difficult, but also exceedingly expensive. Lastly, the ability of pneumococci to change their capsular serotype as a result of uptake of heterologous DNA suggests that the protective effect of anticapsular antibody may be all too temporary, as vaccine serotypes "deliberately" modify their surface polysaccharide in response to mucosal antibodies.

All of these considerations lead to the conclusion that new generations of pneumococcal vaccines will be developed to address these problems. Just as the capsular polysaccharide vaccines have now given way to the polysaccharide-protein conjugates, the future predicts that pneumococcal surface proteins, found to be immunogenic and conserved among global serotypes, may be employed either as the protein component in conjugate vaccines or as a single immunogen. The use of such proteins as immunizing antigens might serve not only to prevent colonization in fully immunized hosts, but also to ameliorate the effects of breakthrough infections in incompletely protected populations such as infants. Work in several laboratories has shown that several different pneumococcal surface proteins (pneumococcal surface protein A [PspA], pneumococcal surface adhesin A [PsaA], and autolysin) are able to elicit active protection against pneumococcal infection in mice when challenged with lethal doses of pneumococci. More recent data strongly indicate that antibody to PspA in human serum can protect mice from fatal pneumococcal infection. As a result of these studies, PspA is now being developed as a human vaccine by a major vaccine manufacturer and is now in phase I trials. In the future, it is likely that many of these common pneumococcal proteins will also serve as useful carriers for inducing antibody responses to weakly immunogenic proteins and polysaccharides. In addition, other new and unique pneumococcal constructs are being developed that will induce much stronger antiprotein and antipolysaccharide immunity following either subcutaneous or intranasal immunization and even, perhaps, bypass the need for T cells and adjuvant.

Studies in other laboratories looking at virulence factors in addition to the polysaccharide capsule have identified pneumolysin as a major pneumococcal toxin. Pneumolysin has a unique role in pneumococcal infection as it allows the bacteria to breach the tissue and mechanical barriers that otherwise confine infection to the respiratory tract. Consequently, pneumolysin is the principal means by which pneumococcus can disseminate from the lung into the blood and cause lethal infection. Because pneumolysin can induce good antibody responses, it is conceivable that future pneumococcal vaccines incorporating pneumolysin may be more effective at preventing invasive infection than the currently available licensed vaccines. In this regard, pneumolysin may serve as an excellent carrier protein for future conjugate vaccines by providing additional protection to pneumococcal serotypes not included in the conjugate formulation. Preclinical studies with one such tetravalent conjugate vaccine containing 6B, 14, 19F, and 23F serotypes demonstrated both high-titered IgG ELISA (enzyme-linked immunosorbent assay) antibody responses and functional (i.e., opsonophagocytic) antibody activity to both the pneumolysin and the various capsular polysaccharides. The antibody response to each of the pneumococcal serotypes was comparable to that observed in animals immunized with a tetravalent conjugate vaccine containing the same serotypes and a tetanus toxoid carrier.

Additional studies have revealed that interferon-gamma, a cytokine that regulates macrophage activity, is an important component of the early host defense against invasive pneumococcal infection. Serum concentrations of interferon-gamma rise in proportion to the virulence and degree of bacteremia produced by the pneumococcal strain in mice. Most importantly, animals that are rendered genetically incapable of producing interferon-gamma are less able to clear pneumococci from lungs and blood and are more susceptible to lethal pneumococcal infection. These studies suggest that the early mortality from invasive pneumococcal infection, which is largely unaffected by antibiotic therapy, might be reduced by augmenting host defenses through the administration of exogenous interferon-gamma at critical time points.

Additional exciting data have demonstrated that intranasal immunization with pneumococcal antigens can lead to protection against pneumococcal disease, and more importantly, against pneumococcal carriage in the nasal passages of mice. This discovery may be critical to the eventual control of pneumococcal disease. Pneumococci are spread by person-to-person contact. They are found in the nasal passages of between 10 and 50 percent of humans (depending on their age and health status). In most cases, carriage does not result in disease, but in some cases the pneumococci invade from the nasal tissue to cause pneumonia, ear infections, eye infections, or meningitis. Vaccines that could prevent carriage would, theoretically, be able to prevent the spread of pneumococci and ultimately its ability to cause disease.

The past decade, which has successfully exploited many of the advances in basic science research and applied them to the field of vaccines, has also uncovered a number of potential obstacles, including

- Accumulating data that suggest that coadministration of different vaccines may be less effective than administration of the individual vaccine components.
- Indications that priming with protein carriers has had variable effects on subsequent immunization with protein-polysaccharide conjugate vaccine. Such priming has been shown both to enhance as well as suppress the response to the polysaccharide depending on the chemical and physical characteristics of the conjugate, timing of injection, and age of administration.
- Improvement in delivery systems and in adjuvants that has demonstrated a tremendous heterogeneity in vaccine efficacy, with some adjuvants enhancing the responses to some immunogens, while having no effect on the response to others.
- Accessibility of vaccines to developing countries due to technological expertise requirements (e.g., conjugation of proteins to polysaccharides).
- Vaccines that are effective in neonates are not necessarily as effective in a geriatric population (e.g., conjugate vaccines) or in a T cell-deficient HIV population.

- Many effective vaccines are not suitable for mucosal immunization, which may prove to be a useful route of administration.

To overcome many of these problems, new novel approaches have been designed, including the use of microencapsulated antigens, DNA vaccines, live vector vaccines, and the use of relevant cytokines to duplicate the physiologic immune system. These new approaches will allow, it is hoped, the development of biological systems that may lead to new generations of pediatric and geriatric vaccines for *S. pneumoniae.*

For reasons that are incompletely understood, the overwhelming majority of penicillin-resistant multidrug-resistant *S. pneumoniae* isolates express a select few of the 90 different capsular types associated with the pneumococcus. These capsular types were predominantly types 6B, 9V, 14, 19F, and 23F. The restriction of these dangerous drug-resistant bacteria to such few serotypes raised the hopes that appropriate conjugate vaccines, which include these few serotypes, could corner the most dangerous strains of *S. pneumoniae.* Recent work led to the discovery by which resistant bacteria could break out of this "corner." The process appears to involve the acquisition of DNA molecules by multidrug-resistant strains (i.e., DNA molecules released by other pneumococci), which carry genetic determinants of new capsular types. In a recent outbreak of multidrug-resistant pneumococcal disease among AIDS patients in New York, a most unusual phenomenon occurred—the appearance of a widely spread multidrug-resistant pneumococcal strain that acquired the capsular type 3 (this strain usually expresses the 23F). This bacterium was resistant to all antibiotics currently used against pneumococci except vancomycin. A simple test using a mouse model showed that these capsular type 3 "transformants" of the multidrug-resistant pneumococcus have increased their virulence capacity more than a millionfold over that of the same bacterium when it carries the usual 23F capsule. Wide-scale deployment of pneumococcal vaccines may produce a selective pressure for this type of capsular switch among clinical isolates. The above finding emphasizes the importance of increased international surveillance for resistant pneumococci.

The most common use of antibiotics is the treatment of respiratory tract infections. The specific etiologic agent or agents responsible for such infections are seldom identified and, therefore, any antibiotic would need to target the major pathogens of this site: *S. pneumoniae, Mycoplasma pneumoniae,* and *Haemophilus influenzae.* Because these represent different classes of organisms, such agents in the past were broad spectrum, resulting in undesired effects on the normal flora and promoting the acquisition of resistance. A common mechanism among these bacteria required for colonization of the human respiratory tract would be an ideal target for a highly specific antibiotic. Such an agent could be effective if it failed to kill the pathogen by effectively interrupting the ability of the organism to interact with its human host.

Recent work has described a genetic locus present in all strains of *H. influenzae,* which decorates its lipopolysaccharide with the host-like structure phosphorylcholine (ChoP). This highly unusual bacterial structure is also found on the pneumococcus and has recently been identified on the surface glycolipid of mycoplasma. The ChoP structure appears to be critical for the viability of *S. pneumoniae* and the ability of both *H. influenzae* and *S. pneumoniae* to be carried within the nasopharynx. The importance of ChoP in mycoplasma has not yet been addressed. Choline is not described in other species of medical significance with the exception of a few members of the genus Clostridia. The key gene, *licA,* appears to be a choline kinase, based on the presence of the putative reactive site for choline kinases in eukaryotes. This gene is also present in mycoplasma, and a similar sequence has been identified in pneumococcus by polymerase chain reaction. Other than the active site, there is no significant sequence homology between the bacterial choline kinases and the eukaryotic choline kinases (including human). This bacterial choline kinase would be a rational target for novel antibiotic therapy aimed specifically at treating or preventing respiratory tract infection due to *S. pneumoniae,* mycoplasma, and nontypeable *H. influenzae.*

Bacteria transit from the nasopharynx to the lung, blood stream, and meninges by using adhesive proteins to bind to human cell receptors. Five adhesive proteins, the first ever described for pneumococcus, have been cloned, sequenced, and demonstrated to convey adherence for the bacteria. The regulatory mechanism affecting which adhesin is presented when in the growth cycle of the bacteria has also been identified. Surprisingly, the ability to stick to human cells is regulated together with the ability to take up DNA for transformation (which is key to developing antibiotic resistance) and with the ability to lyse in stationary phase (a process downregulated in antibiotic-tolerant bacteria). Thus, a clear-cut program has been charted as to how the pneumococcus enters and travels throughout the human host. A key aspect of the mechanism of this targeting is the presence, on the bacterial surface, of choline, an important constituent of human lung fluid and of the inflammatory mediator, platelet-activating factor (PAF).

Translating this information to medical therapeutics has been undertaken. Antagonists of PAF receptor are available, and when deployed in animals with pneumonia, the disease is cleared as effectively as with antibiotics. Furthermore, using an excess amount of the sugars that pneumococci bind to on human cells washes the bacteria out of the lung. These properties suggest very different ways to improve the outcome of disease that do not involve killing bacteria and suggest ways to stop colonization from developing into disease and could be used to prevent the spread of virulent strains. Finally, the ultimate goal of understanding the participants in the trafficking of bacteria is the development of a pool of proteins from which a pneumococcal vaccine can be developed. This possibility has become much more tangible as the pool now stands at approximately a dozen highly protective candidate antigens.

The three major pathogens of children—pneumococcus, meningococcus, and *H. influenzae*—share a set of unusual characteristics. They colonize the nasopharynx and cause respiratory, vascular, and meningeal infections; they are naturally transformable; and they lyse and die in stationary phase. It is now clear that they also share choline on their surface, which contributes to the ability of these pathogens to thrive in the human respiratory tract. Work over the past year has suggested that this "formula" defines a highly regulated and successful program for pulmonary infection, which is driven by the biology of lung fluid choline. Understanding the control mechanism and the proteins that participate in binding to the lung is a big step toward designing a

pneumococcal vaccine and to finding nonantibiotic, novel ways to interrupt the development of pneumonia.

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Sexually Transmitted Diseases

Overview

The human immunodeficiency virus (HIV) pandemic has focused attention on sexually transmitted diseases (STD), both because HIV infection is a fatal STD and because other STDs such as chancroid, genital herpes, syphilis, trichomoniasis, gonorrhea, and chlamydial infection have been implicated repeatedly as risk factors for the sexual transmission of HIV. It is now clear that the risk of becoming infected, or infecting others, with HIV is increased substantially if one has an STD. More than 75 studies on the role of STDs in HIV transmission have been conducted; in 15, both ulcerative and nonulcerative STDs increased risk of HIV transmission approximately threefold to fivefold, independent of the effect of sexual behavior. Although the risk of transmission of HIV in the genital ulcer diseases appears to be higher than in the discharge diseases, the high prevalence of discharge diseases results in a much higher population-attributable risk. A recent study in Mwanza, Tanzania, demonstrated that syndromic management of discharge diseases in men correlated with a 40 percent decrease in HIV infection. Implementation of programs to control STDs is, therefore, a logical next step in preventing the spread of HIV infection.

The control of STDs also is very important in HIV-infected people since more severe disease symptoms may enhance the infectivity of HIV. Recent studies in Malawi have demonstrated that treatment of gonorrhea coinfection in HIV-infected men significantly decreases HIV shedding in ejaculate. More than 80 reports on the impact of HIV infection on STDs suggest that, at a community level, HIV infection may increase the prevalence of some STDs (e.g., genital ulcers). If coinfection with HIV prolongs or augments the infectiousness of individuals with STDs, and if the same STDs facilitate transmission of HIV, these infections may greatly amplify one another. This "epidemiological synergy" may underpin the explosive growth of the HIV pandemic in some populations.

Apart from the HIV epidemic, STDs cause significant morbidity and mortality, as well as contribute greatly to increasing health care costs. Furthermore, STDs disproportionately affect the female, the fetus, and the newborn. Gonococcal and chlamydial infections cause pelvic inflammatory disease, infertility, and ectopic pregnancy. Several common STDs adversely affect pregnancy and result in spontaneous abortion, stillbirth, chorioamnionitis, premature rupture of membranes, preterm delivery, and postpartum endometritis. Neonatal infections include gonococcal conjunctivitis, which may lead to blindness; chlamydial pneumonia, which may lead to chronic respiratory disease; and herpes encephalitis. Moreover, genital infections attributable to human papillomavirus are causally associated with cervical cancer, the most common cause of cancer-related death in women throughout the world.

Despite recent global efforts in health education aimed at preventing the sexual transmission of HIV, STDs remain hyperendemic in many developing countries and in the inner-city populations of industrialized countries. Throughout the world, the majority of STDs are clustered in the resource-limited settings of urban and peri-urban areas where increasing numbers of adolescents and young adults, poverty, unemployment, lack of education, inferior status of women, and social disintegration fuel the epidemic spread of STDs.

A consensus has emerged that the prevention of sexually transmitted HIV infection and the prevention of the major sequelae of STDs in women and infants mandate a global initiative for the prevention and control of STDs. Among other things, this initiative will depend on the development of safe, effective vaccines that prevent infection, disease, and/or sequelae. Currently, except for hepatitis B virus (HBV) infection, no such vaccines exist.

Gonorrhea

Microbial Strategy: As an obligate pathogen of humans, the gonococcus has evolved to effectively avoid, subvert, or ignore the immunodominant host response using strategies that include:

- Phase variation: the ability to turn on or off the synthesis of a surface antigen, e.g., pili and opacity protein (Opa);
- Antigenic variation: the ability to synthesize a particular antigen from a large repertoire of antigenic types, e.g., pili, Opa, and lipopolysaccharide (LPS);
- Surface microheterogeneity: the ability to vary surface immunoaccessibility of antigens among organisms within a

population; this appears to be characteristic of all gonococcal surface antigens;
- Elicitation of immunodominant responses that are not protective: the ability to present surface immunogens, the response to which is not protective, i.e., the generation of antibodies to the reduction modifiable protein (Rmp) that block the bactericidal activities of other antibodies; and
- Enzymatic alteration (sialylation) of gonococcal LPS by the host's enzymes; correlates with resistance to serum bactericidal activity.

Vaccine Strategy: Taking these observations into account, it is very likely that multiple immunogens will be included in an effective vaccine. Furthermore, protective immunogens undoubtedly will have one or more of the following characteristics. They should

- Be microbiologically essential throughout the life cycle, i.e., be expressed constitutively;
- Be phenotypically invariant within and between strains;
- Elicit bactericidal and/or opsonic antibodies;
- Induce functional immunity at the mucosal surface of the reproductive tract; and
- Not be contaminated with Rmp.

Several stable surface components have been identified. Genes for both types of the outer membrane porin protein (Por) have been cloned and sequenced, and common epitopes have been identified. Some epitopes elicit bactericidal antibodies. Chimeric genes encoding combinations of these epitopes have been expressed in *Escherichia coli*; such constructs are potential recombinant vaccines. Corresponding synthetic peptides also are being used as immunogens.

In wild-type strains, Rmp copurifies with Por; these heterogeneous antigenic preparations have elicited Rmp-specific blocking antibodies that interfere with the activity of Por-specific bactericidal antibodies. To circumvent this problem, the Por protein has been purified from genetically engineered mutants that lack the *rmp* gene. Purified Por has been incorporated into liposomes and screened for immunogenicity and protection in animal models. These preparations elicit bactericidal and opsonic antibodies. Phase I clinical trials using purified Por antigen will be conducted in the STD Clinical Trials Unit in 1998.

Three other components have been shown to be potential vaccine candidates on the basis of surface exposure, common epitopes, and the bactericidal action of antibodies directed at these targets. These are iron-binding proteins, proteins expressed anaerobically, and a lipoprotein unique to the pathogenic Neisseria, the H.8 antigen.

Recently, encouraging results have been achieved using a gamma-irradiated whole-cell vaccine as an immunogen. By combining parenteral priming with oral immunization, bactericidal activity, which is not complement-dependent, has been obtained. Vaccinated mice become very resistant to gonococcal infection when one of two animal models—the subcutaneous chamber model in mice or the estrogen-primed vaginal model in mice—is used. Recent studies have revealed that the basis for immunity is an antigen-induced peptide, similar to the antibiotic peptides, defensins, that have been described in other systems.

Studies on the protective function of LPS-induced anti-idiotype antibodies are under way. A conserved oligosaccharide epitope, expressed both *in vitro* and *in vivo*, has been identified. This epitope is not similar to the common gonococcal LPS/red blood cell antigen. When rabbits and mice are immunized with the anti-idiotype to this epitope, serum bactericidal antibodies are elicited.

Using the human challenge model to study the pathogenesis of urethritis, investigators have determined that mutants lacking the transferrin receptor are unable to establish infection, suggesting that this protein might be an effective vaccine candidate. In this model system it has recently been demonstrated that volunteers reexposed to the same strain of *N. gonorrhoeae* are susceptible to reinfection, indicating that short-term exposure, similar to disease in a symptomatic male treated shortly after infection, does not confer immunity.

Chlamydial Infection

Progress toward vaccine development has been aided by the realization that there are two types of immune responses to chlamydial antigens; one is protective (in an *in vitro* model) whereas the other, a deleterious component, is probably an integral part of the development of scarring, the hallmark of chronic chlamydial disease (demonstrated in a guinea pig model). Work is progressing on the molecular basis of these two responses; current efforts reflect the hypothesis that the chlamydial major outer membrane protein (MOMP) elicits neutralizing antibodies, whereas the heat shock protein elicits antibodies that correlate with—or perhaps mediate—scarring and chronic disease. Women with high titers of serum antibody to the chlamydial heat shock protein are at substantially higher risk for acute pelvic inflammatory disease if infected with *C. trachomatis*. Furthermore, HLA class II alleles DQA*0401/DQB*0402 are associated with high titers of antibodies to the heat shock protein, suggesting that host genetics may influence the development of chronic sequelae. Another heat shock protein, hsp10, has been associated with delayed hypersensitivity in the primate model.

Much attention is directed at developing strategies that will selectively stimulate a protective immune response. Antibodies to MOMP have been shown to block binding of infectious particles to host cells and to protect mice from death following injection of viable chlamydia. The MOMP genes from several serovars have been isolated and sequenced; a number of common regions and variable regions have been identified. Recently, vaccine experiments in monkeys, using a chimeric peptide containing both B and T cell epitopes of MOMP, have demonstrated production of neutralizing chlamydia-specific antisera. However, in a murine model of chlamydial infection by a human strain, an anti-idiotypic antibody provided protection against challenge with the organism; protective immunity to chlamydial infection was associated with antibodies targeted to exoglycolipid antibody but not to antibodies targeted to MOMP antigen.

Current efforts are focused on eliciting a protective *mucosal* immune response. Strategies include delivery of antigen by alternative routes and alteration of vaccine formulations. Manipulation of lymphocyte trafficking is also being pursued as a creative approach to invoking mucosal responses with parenteral immunization.

Genital Herpes

Genital herpes is caused by herpes simplex viruses 2 and 1 (HSV-2 and HSV-1). It is estimated that between 40 and 60 million Americans are infected. Each year in the United States, there are 500,000 new infections and 10 million recurrences. As with other herpesvirus infections that involve the nervous system, the lesions are often extremely painful, and the psychological trauma and depression are often serious consequences for infected adults. The disease is insidious in that 80 percent of infected people have either no or mild clinical symptoms and are unaware of their infection; yet, they can reactivate and transmit the virus. Recent studies have demonstrated that people shed virus up to 20 percent of the days of each month. There are two fatal consequences of genital herpes: the transmission of infection to neonates at delivery and the acquisition of HIV infection, as discussed above. In neonates, unlike adults, infection is generally symptomatic and severe. Affected infants (approximately 1,500 in the United States each year) have almost a 50 percent risk of death or severe, permanent neurologic damage.

Herpes simplex virus vaccines have been refined dramatically since the 1920s, when patients were injected with untreated vesicular fluid. Recently, vaccines consisting of recombinant protein subunits, plasmid DNA, replication-defective viruses, and novel adjuvants have been used in human trials; several approaches are in development. No herpes vaccines are yet licensed for use in humans. The need is urgent; in the 17 years since HIV prevention efforts began, HSV-2 seroconversion rates, now estimated at 2 million annually in the United States, have increased 70 percent. Of the 2 million incident infections, 600,000 manifest as clinical disease. Transmission of HSV is probably driven by the large reservoir of asymptomatic carriers, who frequently shed infectious virions. It is estimated that 40 to 60 million Americans are infected.

Until recently, patients were enrolled in two phase III trials of herpes simplex vaccines. Both trials were designed to test the efficacy of recombinant subunit vaccines, one developed by Chiron containing two glycoproteins, and the other by SmithKline Beecham containing one glycoprotein. Both vaccines consist of viral coat proteins produced in a recombinant bacterial system, together with an adjuvant. The vaccines utilize two different adjuvants—Chiron uses MF59, whereas SmithKline uses monophosphoryl lipid A immunostimulant (MPL), a bacterial product obtained in partnership with Ribi ImmunoChem Research. Chiron halted its trial because the results seen in phase I and II trials were not replicated in the preliminary results of the phase III trial. SmithKline continues to enroll patients, in the hope that the single glycoprotein and the cell wall adjuvant MPL will lead to an improvement in efficacy. The SmithKline vaccine actually employs several approaches to enhancing immunogenicity—the recombinant protein is first precipitated on alum, then suspended in an oil-in-water emulsion containing MPL. The results of the SmithKline trial should be released in 1998.

The newer-generation vaccines, which often involve the introduction of engineered DNA into the human body, are beginning clinical trials. Some of these use traditional viral vectors, while others, termed DNA vaccines, employ naked plasmid DNA. Furthest along of the early investigational vaccines is the DISC (disabled infectious single cycle) herpesvirus vaccine, which is being developed by Cantab Pharmaceuticals. Subjects participating in the phase I trials are vaccinated with replication disabled HSV-2 virions that are indistinguishable from wild-type virus except for one protein. The difference is that the gene for glycoprotein H (gH), necessary for viral entry into the cell, has been deleted from the virus' genome. To compensate for this deficiency in the first replication cycle, the disabled virus is provided with the missing protein by growing it in monkey kidney cells transfected with the missing gene. The virus takes along only the protein product, not the gene for producing it, which remains in the kidney cells. The DISC herpes vaccine is being tested in the United States and the United Kingdom. This phase I study will form the basis for Cantab's therapeutic vaccine trial, and the results of this trial are expected this year. In phase II, the prophylactic efficacy of the vaccine will probably be tested in seronegative partners of discordant couples, at risk of contracting genital herpes.

A DNA vaccine made by Apollon began phase I trials in September 1996. A total of 40 healthy HSV-2-seronegative volunteers—including 20 who are seropositive for HSV-1 and 20 seronegative—will be enrolled in this ongoing double-blind controlled trial, designed to determine safety and begin to measure immunogenicity. Further clinical trials, this time in HSV-2-seropositive individuals, are soon to be conducted at the University of Washington. Enrollment is already being solicited in area newspapers. The vaccine consists of plasmid DNA, which encodes glycoprotein D2 (gD2). It contains no adjuvant, but a lipid is included as a "facilitator" to increase uptake of plasmid DNA into the vaccinee's muscle cells. Like gH, gD2 is necessary for viral entry into cells and is known to be one of the more immunogenic of the 75 gene products produced in the herpes-infected cell.

Virus Research Institute is currently developing a vaccine based on a replication-defective mutant of HSV-2, 5BlaZ, which contains an ICP8 gene mutation. Investigators at Harvard Medical School, originators of this vaccine strain, have recently shown that immunization with this mutant virus protects guinea pigs from primary as well as recurrent disease following challenge with virulent HSV-2.

Pharmadigm, Inc., has developed a proprietary DNA vaccine construct containing a muscle-specific promoter and the gD2 gene. They have made a vaccine using this construct and 1,25-dihydroxyvitamin D (1,25-D3) as an adjuvant. In rodent models, the plasmid induces production of gD2 in muscle cells. Pharmadigm has found, using a mouse model of primary HSV-2 infection, that when 1,25-D3 is injected along with the recombinant plasmid, the combination enhances protection from severe disease. The company also plans to experiment with dihydroepiandrosterone (DHEA) as an adjuvant; this is a precursor to many androgens and has been shown to restore antigenic responsiveness to stimulated senile immunocytes. Another innovation in the Pharmadigm DNA vaccine is the choice of plasmid. Researchers there achieved controlled expression of the gD2 gene with a muscle-specific promoter activated by myoD, a protein expressed preferentially in myotubes. It is hoped that this will alleviate the concern that nonspecific viral promoters might activate systemic tumor-inducing genes or other adverse genes. The company is currently modifying its plasmid to align with Food and Drug Administration regulations and is looking for corporate sponsors to help bring its technology to human trials.

Researchers at the Children's Hospital Medical Center in Cincinnati, Ohio, have evaluated a plasmid DNA vaccine pro-

duced by Vical. Their vaccine, which so far has only been tested in guinea pigs, uses an immediate-early cytomegalovirus gene promoter to express gD2. No component of this vaccine is separately classified as an adjuvant. However, it is believed that the plasmid DNA itself may induce a more rigorous response to the foreign gene product.

Scientists working at Cel-Sci and collaborators at Northeastern Ohio Universities College of Medicine have developed a group of peptides that theoretically work to preferentially stimulate cellular immunity, the immune response that is considered most likely to combat HSV effectively. The vaccine will make use of Cel-Sci's new heteroconjugate technology, in which small disease-associated peptides will be linked to T cell binding ligands. The ligand theoretically presents the peptide to certain classes of T cells to induce specific immunity to fight herpes infections. The ultimate goal will be to develop a vaccine that protects the individual without the potential dangers of an attenuated virus or a DNA vaccine. The group is now using mice as a model to test its first batch of HSV-peptide heteroconjugate candidates.

Genital Warts and Cervical Cancer

In the United States, it is estimated that between 28 million and 40 million people are infected with human papillomavirus (HPV). HPV genital infections are associated with anogenital cancer, in particular with cervical cancer, one of the most common causes of cancer-associated death in women in the developing world and a cancer that kills 4,800 American women annually. Routine Pap smear screening is widely credited with reducing cervical carcinoma from the number one to the number eight cause of cancer death in American women, but the costs of providing Pap screening are considerable (estimated at $6 billion annually for American women). In addition, adequate screening is not available for all women, even in the United States. In developing countries, in the absence of screening programs, cervical cancer causes 250,000 cancer deaths in women each year.

The prevalence of HPV infection among sexually active women may range from 18 to 25 percent, especially in some populations of sexually active teenagers. Although most of these infections will not progress to cancer, many will cause cervical abnormalities. In each case, however, these women may transmit HPV to their partners or to their babies. In the neonate, HPV infection infrequently leads to warts in the oral cavity and upper respiratory tract. In HIV-infected immunocompromised adults, HPV infection appears to cause severe and rapidly progressing disease. This reinforces the evidence-based belief that the immune system plays a key role in ameliorating disease, if not preventing infection.

The disease rate for genital warts, also caused by HPV, is estimated to be 1 million Americans per year. Genital warts are sometimes difficult to treat; current treatment modalities (freezing, burning, and laser surgery) are associated with a 20 to 50 percent recurrence rate.

Vaccines against HPV—both the high-risk strains commonly associated with cervical cancer and the low-risk strains associated with genital warts—are a priority for a number of pharmaceutical and biotechnology firms. Two companies currently conducting clinical trials for these vaccines are Cantab and MedImmune. Merck has produced a vaccine that will enter human trials in 1998. Apollon is also developing an HPV vaccine but has not begun clinical trials.

Cantab is developing three vaccines for the treatment or prevention of HPV-related disease. TA-HPV, for immunotherapy of cervical cancer, and TA-GW, for immunotherapy of genital warts, have demonstrated safety and immunogenicity in phase I and II clinical trials. TA-CIN, for the treatment of patients with cervical dysplasia, is in preclinical development.

TA-HPV is a live recombinant vaccinia virus engineered to express the E6 and E7 genes from HPV types 16 and 18, the principal viruses associated with cervical cancer. E6 and E7 proteins are believed to be involved in transformation of HPV-infected cells. The results of a phase I/II clinical trial of TA-HPV, conducted at the University of Wales College of Medicine, Cardiff, U.K., and published in a June 1996 issue of *Lancet* were encouraging. The study, which successfully demonstrated that the vaccine causes no significant side effects, is paving the way for future studies of the vaccine's clinical efficacy. The clinical efficacy of TA-HPV could not be determined in the initial trial because the study group was too small and involved only patients with advanced disease. A followup trial using multiple doses on patients with less advanced disease has begun.

TA-GW is undergoing clinical trials in males with genital warts. TA-GW is a recombinant fusion protein made up of the L2 and E7 proteins of HPV type 6, produced in *Escherichia coli* and formulated onto alhydrogel adjuvant. There is marked homology between the proteins of HPV types 6 and 11, which may lead to immune cross-reactivity against type 11. (HPV types 6 and 11 are the principal viruses associated with genital warts and laryngeal papillomatosis.) The L2 protein makes up around 5 percent of HPV's viral coat. The vaccine has been shown to induce a serum IgG response in humans, as well as cellular immune responses, demonstrated by *in vitro* lymphocyte proliferation responses.

In December 1996, Cantab announced the conclusion of a phase IIa open-label trial using the TA-GW vaccine. The vaccine was used in 27 male patients, 16 with recurrent and 11 with new genital warts. The vaccine was given in three intramuscular injections at 0, 7, and 28 days. At week 8, six patients showed complete clearance. Of the 15 patients followed after week 8, 13 showed complete clearance of warts. So far, none of the patients who cleared the warts have relapsed. (In contrast, patients with genital warts have a 20 to 50 percent chance of recurrence after treatment with existing therapies.) Two other phase IIa trials of the vaccine—one using TA-GW in conjunction with cryotherapy and the other using the vaccine to treat laryngeal papillomatosis—are ongoing.

In July 1996, Cantab entered into a collaboration with SmithKline for the continued development and marketing of its therapeutic TA-GW vaccine for genital warts. The first SmithKline TA-GW-derived vaccine is under development, but the adjuvant has yet to be selected.

MedImmune's prophylactic vaccine for genital warts, MEDI-501, takes a different approach to vaccine development. The vaccine consists of recombinant HPV-11 L1 protein with an alum adjuvant. Recombinant L1 has the useful property of self-assembling into virus-like particles. This property is exhibited by the protein used in hepatitis B vaccine as well. Virus-like particles contain no viral DNA and are noninfectious, but when viewed through an electron microscope, they look very much like virus particles.

More importantly, the particles stimulate production of antibodies that bind and neutralize infectious virus. In a *Proceedings of the National Academy of Sciences* paper published in December 1995, MedImmune investigators described a similar vaccine's performance in beagles. All seven beagles immunized with a vaccine made from canine oral papillomavirus (COPV) L1 protein were protected when exposed to a wart homogenate applied to excoriated buccal mucosa. Control animals injected with detergent-denatured L1 protein were not protected, indicating that the higher-order structure of L1 is important for protection.

MedImmune began phase I trials of MEDI-501 on February 3, 1997. By using healthy volunteers, MedImmune hopes to establish the vaccine's safety and immunogenicity in this year-long, placebo-controlled, dose-escalating trial.

Merck is also preparing a vaccine for clinical trials to prevent genital warts and cervical cancer. Like MedImmune's product, this vaccine uses recombinant proteins that self-assemble into virus-like particles. Merck is working with CSL (Australia) on a quadrivalent vaccine for HPV types 6, 11, 16, and 18—this is the only vaccine that uses recombinant protein from so many different strains of HPV. Preliminary experiments have found that recombinant L1 produced in the yeast *Saccharomyces cerevisiae* formed virus-like particles and, when formulated as a vaccine with an alum adjuvant, the virus-like particles efficiently protected rabbits from challenge with cottontail rabbit papillomavirus.

A group of investigators at the National Cancer Institute has entered into an agreement with the National Institute of Allergy and Infectious Diseases for phase I safety testing of an HPV virus-like particle L1 vaccine. Early results in preclinical animal models have been promising.

Investigators at Johns Hopkins University in Baltimore have developed an entirely distinct approach to a therapeutic vaccine for HPV-derived cervical cancer. They have engineered a vaccinia virus construct containing the transforming proteins of the oncogenic strains of HPV. The construct also contains a molecular signal that routes this protein antigen to the intracellular pathway that generates tumor immunity. These investigators have entered into an agreement with Pasteur Merieux Connaught for the development of vaccines based on this technology.

Merck is also developing an HPV vaccine using an entirely different technology—the naked plasmid DNA. In DNA vaccines, sequences from the viral genome are spliced into a plasmid that controls their expression. Efforts at creating a plasmid DNA vaccine for HPV are still in the preclinical stages. It is thought that delivery of a combination of capsid proteins as plasmid DNA would simplify the preparation of a multivalent HPV vaccine.

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Viral Hepatitis

Overview

The good news is that universal immunization of infants against hepatitis B is increasing yearly in this country, and the Centers for Disease Control and Prevention (CDC) reported that over 60 percent of children born in 1993 were vaccinated. However, recent newspaper articles demonstrated that the U.S public remains at risk of contracting viral hepatitis in spite of the existence of licensed vaccines for some of the causes. Strawberries infected with hepatitis A virus turned up at school lunch programs

in six States. Also, with caseload for hepatitis C on the rise, a consensus conference for clinicians was held last March to determine how to treat patients at different stages of their infection. One of the staggering statistics to come out of that meeting was the future projection of 24,000 deaths per year due to hepatitis C infection in the next two decades.

Five different viruses stand as the known etiologic agents for hepatitis leading to fatigue, jaundice, liver damage, and in chronic cases, cirrhosis and even liver cancer. Hepatitis A is transmitted fecal-orally, and outbreaks of this acute infectious agent are common at day care centers, nursing homes, and restaurants where careless food handling might occur. Hepatitis B and C, both blood borne and both causative agents for chronic diseases, are distinctive viruses—B being far more detectable and symptomatic yet more easily resolved in adult infections. Hepatitis C, with a high chronicity rate at all ages, is far more occult, as those infected may remain asymptomatic in spite of ongoing liver disease. To date, the only licensed therapy for either B or C is interferon-alpha, which exhibits low success rates for both diseases. Infection by hepatitis D is dependent on coinfection by hepatitis B and may lead to life-threatening superinfections. Hepatitis E, like hepatitis A, is transmitted via fecal-oral route and causes an acute clinical picture. It is reported primarily in developing countries and is highly fatal to pregnant women. Surprisingly, however, the CDC has determined by serologic screenings that over 1 percent of the U.S. population has been exposed to hepatitis E.

Several notable advances were made in structural biology of hepatitis viruses this year. The structure of the core protein of hepatitis B, a molecule that does not crystallize and was therefore incapable of being studied by x-ray crystallography, was determined by electron cryomicroscopy by two groups. Two other groups published reports on the x-ray structure of the hepatitis C nonstructural NS3 protease, important in viral replication and now a target for antivirals. At least five companies are presently working on inhibitors for the HCV protease. Finally, researchers have just identified the structure of the helicase enzyme of hepatitis C that is needed to uncoil the viral RNA to allow it to make a copy of itself for reproduction.

Hepatitis A (HAV)

Hepatitis A accounts for 33 percent of acute hepatitis cases in the United States, with the highest incidence in the Southwest. The average incidence of infection is 132,000 cases a year, with elevated numbers among American Indians and Hispanics, people of low socioeconomic levels, and those practicing risky lifestyle behaviors. Rates in males are 20 percent higher than in females, and prevalence of exposure (antibody to HAV) ranges from 11 percent in persons under 5 to 74 percent in persons over 50. Most clinical cases are seen in 10- to 30-year-old patients. Symptoms are nonexistent below the age of 2 but increase dramatically with age. Fulminant disease can be fatal and accounts for 70 to 80 deaths a year among those between the ages of 30 and 49. Work loss costs associated with acute disease in the United States are $200 million each year. Natural immunity levels in the United States have undergone a significant decline since 1980 and are currently in the 21 to 33 percent range. Two formalin-killed licensed vaccines are available for adults and children over 2 years old—Havrix (SmithKline Beecham Pharmaceuticals) and Vaqta (Merck & Co.). Both contain inactivated viral particles (HM175 and CR326F strains, respectively) produced in infected human diploid fibroblasts. Initially labeled for two doses plus a booster dose, travelers can be significantly protected with a single dose. There are other inactivated hepatitis A vaccines not licensed in the United States. Also, combination hepatitis A and B vaccines are being studied in clinical trials.

As outbreaks of hepatitis A are transient and sporadic, universal recommendations for vaccination in the United States have not been instituted. The costs are not covered by many health insurance policies, and the shots are expensive, taxing to those most at risk. Efforts are ongoing to create an attenuated live vaccine that would be cheaper and easier to administer, but so far no candidate is on the horizon nor is the demand necessarily that pressing. The virus is a slow grower in the tissue cultures used to prepare the killed vaccines though researchers are trying to improve on this. Immune serum globulin can be used therapeutically within 2 weeks of exposure to prevent acute infection; however, timely recognition of exposure is critical. Not only is it much less expensive than the vaccine itself, immune globulin can be used instead of the vaccine right before a trip lasting up to 3 months. Or it can be administered at the same time as the vaccine for added protection. The hepatitis A vaccine is recommended for those already infected with other hepatitis viruses.

Hepatitis B (HBV)

The estimates for chronic hepatitis B remain around 300 million worldwide, with endemic areas seen mostly in Asia and Africa. Hepatitis B is highly contagious and, like hepatitis A, is capable of producing fulminant disease. It is highly transmissible from HBV-positive mothers to their newborns. An average of 25 percent of adults infected become chronic carriers, and 20 percent of those develop more severe liver disease such as cirrhosis or cancer. Perinatal infection of infants has a much higher chronicity rate of 70 percent. Each year, an estimated 20,000 infants are born to hepatitis B surface antigen-positive women in the United States. From 200,000 to 300,000 new HBV infections have been reported in the United States annually this decade. Annually, hepatitis B accounts for 60,000 hospitalizations and 5,000 deaths for a total yearly cost of $800 million a year, excluding the cost of transplantation for end-stage liver disease. It is gauged that there are 1 to 1.25 million chronic carriers of HBV in the United States.

Hepatitis B vaccines were first introduced in the early 1980s as either heat-inactivated or chemically inactivated small envelope viral (S) particles derived from chronic hepatitis B plasma. One vaccine, Heptavax, was licensed by Merck, Sharp, & Dohme in 1981. Subsequently two recombinant vaccines were licensed and used: Recombivax HB (Merck & Co.) and Engerix-B (SmithKline Beecham). These second-generation vaccines are being paired up with other licensed vaccines for infants. Also, new recombinant vaccines containing both preS and S antigens are being developed for potentially increased immunogenicity in persons resistant to protection from the licensed recombinants now being used. Other candidates still under development include a salmonella-vectored vaccine and DNA vaccines that may be more efficient at inducing cytotoxic T cells as well as neutralizing antibodies. Transgenic potatoes genetically engineered to express the hepatitis B S antigen have recently demonstrated evidence of mucosal immunity along

with the previously established systemic immunity when fed to laboratory mice.

Universal infant immunization is highly recommended, the positive effects of which have been seen in Japan and most recently in Taiwan where the overall prevalence rate for children from 1 to 10 years old has decreased from 9.8 percent in 1984 to 1.3 percent in 1994. Cancer incidence in 6- to 9-year-olds dropped from 0.52 percent to 0.13 percent in the same study. Although recently initiated, the positive effects of universal infant immunization (inclusive of hepatitis B immune globulin in cases of seropositive mothers) are gradually rising in the United States. Australia is embarking on a nationwide campaign at the projected cost to the government of A$14 million to immunize all school children against HBV. An added benefit of universal immunization against hepatitis B is the prevention of coinfection by hepatitis D at the same time. Unfortunately, there is presently no protection for hepatitis B chronic carriers against this potentially serious coinfection.

Interferon-alpha remains the only licensed antiviral against hepatitis B infection in the United States. Several antiviral candidates are in phase II/III trials and seem quite promising: lamivudine (GlaxoWellcome), lobucavir (Bristol Myers Squibb), adevofir (Gilead), and famciclovir (SmithKlein Beecham). Another newer compound by Bristol Myers Squibb, BMS200475, is also showing tremendous potential in early phase studies. Unfortunately, escape mutants leading to drug resistance are already detected in patient populations receiving either lamivudine or famciclovir for several months. Recent information on lamivudine discusses liver flare-ups and the return of hepatitis after cessation of protracted therapy. Clearly, like HIV, drug cocktails targeting different viral mechanisms will be needed. One exciting nucleoside contender, L-FMAU, developed by an NIAID grantee and recently tested in the NIAID-contracted woodchuck colony, has not only knocked down viremia to levels detectable only by highly sensitive means, but unlike other nucleoside analogs tested, viremia stays down for extended periods after treatment has stopped. In addition, L-FMAU as yet shows no sign of toxicity. These results are exceedingly new and further testing is planned. The therapeutic vaccine Theradigm™ is not yet licensed. Its function is to induce a cytotoxic T lymphocyte response in chronic patients, helping them to resolve their own disease.

Hepatitis C (HCV)

Infection by hepatitis C accounts for 21 percent of acute viral hepatitis in the United States, with an incidence of 35,000 cases per year (declining over the past decade from 180,000), with about 85 percent of those infected becoming chronic carriers at a total yearly cost of $800 million excluding transplants. Many cases of hepatitis C can be attributed to blood transfusions before 1990 from which an estimated 290,000 Americans were infected. Most cases occur among young adults (injecting drug users), although among older adults over 40 years of age HCV is often the most common cause of acute hepatitis. No risk factor has been identified for 10 to 30 percent of carriers. Each year there are 8,000 to 10,000 deaths and 1,000 transplantations. The current estimate, based on random serologic screenings of more than 21,000 serum samples, is that 3.9 million Americans are chronically infected with HCV—1.8 percent of the population, with higher rates in blacks (8 to 10 percent). Hemodialysis patients and hemophiliacs are exceptionally vulnerable, and noninvasive patient-to-patient transmission has been documented. The World Health Organization estimates that 3 percent of the world's population has been infected and that there are 170 million chronic carriers at risk of developing liver cirrhosis and/or liver cancer.

Several investigators have reported a relatively high efficiency of HCV vertical transmission from mothers who were coinfected with HIV. On the other hand, some major studies in the United States and Europe have failed to demonstrate transmission from HCV-positive mothers while others have provided compelling evidence that transmission occurs. Risk factors for transmission, assumed to occur *in utero*, include a high HCV RNA level in the mother and the presence of specific HCV variants. Results of a recent study in children born to infected mothers associate biochemical features of liver damage (ALT abnormalities) during the first 12 months of life although HCV-associated liver disease is likely to be mild throughout infancy and childhood. Interestingly, multivariate analyses of risk factors for hepatitis C and its effects on interferon therapy, fibrosis, and hepatocellular carcinoma (HCC) somewhat repeat the same risk factors: older age, male gender, and excessive alcohol consumption. Additional factors for cancer are hepatitis B antibody positivity and HCV genotype. There was no relationship between the development of HCC and serum HCV levels.

Research in hepatitis C remains handicapped by the lack of reproducible tissue culture models or a convenient, economical, small animal model for testing vaccines and antivirals as well as for studying the natural history and pathogenesis of this silently transmitted pathogen. Many unsuccessful attempts have been made to develop full-length infectious clones of the hepatitis C genome. Recently, by creating consensus sequences of genotype 1a HCV and injecting the RNA transcripts from these clones into chimpanzees, investigators demonstrated both infection and disease. This positive result helps determine the structure of the HCV genome. This proves definitively that HCV is sufficient to cause disease and provides the basis for future molecular genetic studies and a new approach for development of *in vitro* and *in vivo* models.

Although hepatitis C is the leading cause of chronic viral hepatitis in the United States, the development of an effective vaccine is hindered by extensive genetic and possibly antigenic diversity among the different strains. New variants known as quasispecies arise quickly and frequently, thus allowing escape from neutralizing antibodies and cytotoxic T lymphocytes. Amino acid changes observed frequently in a region of about 27 amino acids, termed the hypervariable region 1 (HVR1), which is located at the amino terminus of the hepatitis C envelope protein E2, are postulated to lead to a viral escape from neutralizing antibodies. The identification of the most variable region of HCV, the HVR-1, as a critical neutralization domain poses a major challenge for the development of a broadly reactive vaccine against HCV. Early vaccine studies in chimpanzees using recombinant envelope glycoproteins showed limited protection upon challenge with the same virus. DNA vaccines are now being tested in chimpanzees using not only envelope but also core protein constructs. Ribozymes, catalytic RNA molecules that bind specifically to target RNA by an antisense mechanism, are being tested as a possible strategy for the treatment of hepatitis C infection.

NIAID has expanded its efforts in both basic and applied research in the hepatitis C arena. Four multidisciplinary cooperative research centers are now funded to work toward the development of models to study viral replication, pathogenesis, and immune responses to this agent—including a clinical component at each site. More independent investigators are also being funded, and an annual workshop is held to foment collaborations and exchange ideas. On the heels of the Management of Hepatitis C Consensus Development Conference held in March 1997, NIAID, in cooperation with a panel of experts in the field, has prepared a strategic plan to expand research in hepatitis C into the next millennium. Recently, the NIAID-sponsored Collaborative Antiviral Studies Group added clinical trials in hepatitis to its studies. This forum allows recruitment of patients from all over the country as well as some foreign sites to participate in clinical trials run in underserved areas—pediatric populations, transplant populations, and populations coinfected with HIV. Protocols to test antiviral combinations are presently under review.

Hepatitis D (HDV)

The prevalence of hepatitis D does not parallel that of HBV, although it is dependent on HBV for its transmission. It is highest in those with repeated percutaneous exposures, including IV drug users and hemophiliacs. It is rarely reported following perinatal transmission (as yet undocumented in the United States). An estimated 70,000 people (4 percent of HBV cases) in the United States have chronic hepatitis D; there are 7,500 infections each year, and about 1,000 people die annually of HDV. There are three genetically different types: type I is found worldwide, II in southeast Asia, and III in northern South America.

Hepatitis E (HEV)

In endemic areas 7 to 17 percent of the population show previous exposure to hepatitis E. Unlike HAV, immune globulin has not prevented infection during outbreaks. HEV, transmitted fecal-orally and usually seen in developing countries or in travelers, can cause fulminant disease with high case fatality rates (15 to 20 percent) among pregnant women. Subsequent studies in pregnant rhesus monkeys failed to show a greater degree of severity than that seen in nonpregnant monkeys and it was not transmitted to their offspring. HEV can also infect and replicate in laboratory rats where antigens are seen not only in the liver, but also in the spleen, peripheral blood mononuclear cells, mesenteric lymph nodes, and small intestine. Efforts are ongoing to develop an infected tissue culture model.

HEV is rare in the United States but does pose a risk to persons who travel overseas to endemic areas. The CDC has developed a mosaic protein enzyme immunoassay, which, based on antibody titers, showed a 3 percent incidence of recent exposure to HEV in a cohort of randomly screened patients in four geographic areas of the United States; none had traveled abroad. A 1.2 percent prevalence of previous exposure among the U.S. population was also determined. There is a high seroprevalence among renal transplantation and hemophilia patient populations.

In NIAID studies conducted at the National Institutes of Health, cynomolgus monkeys were partially or completely protected against infection with hepatitis E by passive or active immunization. Convalescent serum was used for the passive immunization and a recombinant 55 kilodalton open reading frame 2 protein known to induce antibody formation was used for the active immunization. Passive immunization did not protect the animals from infection upon challenge, but the active immunization did. Passive immunity offered considerable protection against hepatitis. These results point the way toward development of a vaccine.

Hepatitis G or Hepatitis GB Virus-C (HGV, HGBV-C)

Much progress has been made in analyzing, sequencing, serotyping, and determining the prevalence of the recently discovered blood-borne hepatitis viruses, HGV and HGBV-C. Discovered by Genelabs and Abbot Labs, respectively, these two viruses are now assumed to be actually different isolates of the same virus. Their genomic sequences are in Genbank (U36380 and U44402). Both are distantly related to another flavivirus, hepatitis C (only 25 percent homology). Studies of stored serum cohorts show these viruses are not new; rather, they have awaited new methods for discovery and detection. They are endemic worldwide, though their clinical picture remains unclear as most carriers are asymptomatic. Cases of fulminant hepatitis have been linked to them; however, they are not generally considered to be a cause of nonA-E hepatitis. Multiple strains of HGBV-C have been found in dialysis patients; the virus is common in transplantation settings and is found in 10 percent of injecting drug users. HGV has been significantly associated with coinfection with certain strains of HCV (types 1a, 1b, and 3), but this additional infection does not seem to affect the patient.

Other Hepatitis Viruses

A few years ago a novel fecal-orally spread hepatitis was named hepatitis F by a French team of researchers. No subsequent publications have appeared about HFV, but a second publication did refer to a novel hepatitis agent being detected from a screening of HEV-infected sera from an epidemic in the Andaman Islands. Four percent of acute cases of hepatitis are currently classified as non-ABCDE,G.

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Human Immunodeficiency Virus (HIV) Disease

Overview

As acquired immunodeficiency syndrome (AIDS) continues to take its toll globally, the development of a safe and effective vaccine against HIV is critical to worldwide efforts to control the epidemic. Although educational and counseling efforts have had some success, it has become evident that prevention activities alone are not sufficient to contain the spread of disease.

Thousands of people continue to be infected with HIV every day: an estimated 3.1 million new HIV infections occurred worldwide in 1996, roughly 8,500 per day. Approximately half of the new infections were in women. New cases of HIV are also disproportionately affecting young people: the majority of new cases worldwide are in individuals under 25 years of age. In the United States, an estimated 40,000 individuals are newly infected with HIV each year.

Given the need for a safe and effective HIV vaccine, President Clinton recently challenged scientists to develop a vaccine by the year 2007. Key to responding to that challenge will be the AIDS Vaccine Research Committee and the AIDS Vaccine Research Center, established by the National Institutes of Health (NIH). The AIDS Vaccine Research Committee, chaired by Dr. David Baltimore, will be critical in helping to stimulate HIV vaccine research and will assist the NIH in developing a comprehensive research program aimed at expediting the discovery and development of a safe and effective AIDS vaccine. By bringing together intramural scientists from across the NIH, the AIDS Vaccine Research Center will stimulate multidisciplinary research from basic and clinical immunology and virology through to vaccine design and production. The Center will complement the comprehensive extramural research activities of the Division of AIDS, National Institute of Allergy and Infectious Diseases (NIAID). [Clinical trials of HIV vaccines, many of which are conducted by the NIAID-supported AIDS Vaccine Evaluation Group (AVEG), are described in detail in Appendix C.]

NIAID also recently unveiled a new grant program called the Innovation Grant Program for Approaches in HIV Vaccine Research, which is designed to speed the pace of AIDS vaccine discovery and development. It will do so by supporting research projects that may involve a high degree of innovation and risk, that show clear promise in improving vaccine design or evaluation, and that bring new investigators into the field of HIV vaccine research.

In addition to the efforts of the NIH are those of other Federal agencies, including the Centers for Disease Control and Prevention, the Department of Defense, the Food and Drug Administration, pharmaceutical companies, and nongovernment agencies. Augmenting all these research activities are the HIV vaccine research activities sponsored by other countries.

There are sound scientific and strategic reasons that support these extensive efforts in HIV vaccine discovery and development.

- Immunization is a well-established method of preventing viral infections.
- Passive transfer of anti-HIV or anti-SIV (simian immunodeficiency virus) antibodies to chimpanzees or monkeys has prevented infection under certain conditions.
- Some vaccines for HIV and related retroviruses have protected against challenge in certain animal models.
- To date, several HIV vaccine candidates in early human trials have been safe and immunogenic.
- Biostatistical modeling suggests that even a partially effective preventive intervention may have a powerful effect on the epidemic.

In addition, as a result of recent scientific advances, many of the obstacles to vaccine development appear to be much less formidable than had been previously thought. For example, it has long been feared that the variation of HIV within populations and individuals would enable HIV to evade specific cytotoxic T lymphocyte (CTL) and antibody responses that may be crucial for protection against HIV/AIDS. Recent studies of HIV receptors have revealed a spectrum of coreceptors, including CCR5 and CXCR4, used by HIV that may eventually form a basis for devising vaccines that can induce broadly neutralizing antibodies. A few monoclonal antibodies and some sera from infected individuals react against a variety of primary isolates of HIV. In addition, new methods for measuring CTLs have shown that CTLs from people infected with one subtype of HIV can recognize target cells representing subtypes from other geographic regions. In addition, mea-

sured against a panel of isolates from around the world, CTLs from vaccinated individuals can often recognize several genetic variants of HIV. This suggests that if CTLs (or other T cell antiviral activities) are critical to controlling or eradicating HIV infection, one vaccine may be able to immunize diverse populations against many different strains of HIV. These new findings suggest that the remarkable genetic diversity of HIV is not an insurmountable obstacle to developing a broadly reactive preventive vaccine.

In the past, there has also been concern that it would be more difficult to prevent HIV infection from sexual transmission as compared to HIV acquired through parenteral transmission (Sabin). Increasing evidence from animal models suggests that there is a short period during which the establishment of chronic infection may be prevented if infection occurs through vaginal, rectal, or oral exposure. Clinical and laboratory methodology for identifying binding antibodies and for cloning CTL from mucosal fluids or cellular specimens may help elucidate the local immune response during mucosal infection. Additionally, genetically altered toxins and physical carriers are also being developed to enhance mucosal presentation, and they may be effective in inducing mucosal immunity in humans. Thus, it may be more feasible than previously thought to prevent sexual or mucosally based transmission through either parenteral or mucosal immunization.

As researchers struggle to overcome these and other scientific challenges, individuals in the communities from which study volunteers will be recruited are working to overcome fears and preconceived notions about vaccine trials. (Volunteers will include both men and women at high risk of HIV infection because of their sexual behavior or drug use.) In addition to potential biologic risks of any new vaccine, there are concerns about the social impact of receiving a candidate HIV vaccine. Because vaccination can induce antibodies against components of HIV, some volunteers may test positive for HIV on standard HIV diagnostic tests. Although the vaccines being tested cannot result in HIV infection, volunteers may fear the social consequences and discrimination of falsely testing positive. NIAID continues to work proactively to minimize the potential for discrimination against study volunteers and over the past several years has developed strong linkages with various communities to address many of their questions and concerns about HIV vaccines. Public health and diagnostic laboratories, insurance companies, and manufacturers of diagnostic kits are helping to address volunteers' concerns.

Investigators continue to design and test novel ways to present HIV proteins to the immune system and evaluate new antigen/adjuvant and various vaccine formulations. Traditional approaches to immunization (live-attenuated, whole-inactivated) are technically complex and raise substantial safety concerns. Clinical trials involving candidates for prophylactic vaccines enroll volunteers who are not infected with HIV. Such trials have been conducted to test vaccines made through chemical synthesis or biotechnology: subunit vaccines including envelope proteins or small particles as well as recombinant poxviruses bearing genes encoding one or more viral proteins and DNA vaccines. To date, the AVEG, which supports the intensive study of novel HIV vaccine candidates in human trials, has enrolled more than 2,300 uninfected volunteers, in 47 studies involving 22 vaccine candidates. Of these, three vaccines have proceeded to phase II trials: two different recombinant gp120 envelope proteins and one recombinant canarypox-HIV vaccine, which incorporates genetic material from the HIV envelope, gag and protease genes.

A brief description of the various types of HIV vaccines being tested and the status of research in each of these areas follows.

Protein and Peptide Subunit Vaccines

Subunit vaccines consist of small protein or peptide portions of pathogenic virus. They can be made by genetically engineering bacteria, yeast, insect, or mammalian cell cultures to produce protein subunit antigens. Envelope subunit and peptide approaches were among the earliest attempts to make an HIV vaccine, based on the premise that the envelope protein would be the most important target since it binds to cells and allows viral entry.

Phase I trials have been conducted with the full-length *env* gene product (gp160) or the envelope protein gp120 produced in insect cells, yeast, or mammalian cells. The highest titer and most broadly reactive neutralizing antibodies were induced by the mammalian gp120 vaccines. However, the first vaccines were based on laboratory-adapted strains of HIV that used the CXCR4 receptor to enter T cells; the antibodies induced by these vaccines do not neutralize primary HIV isolates grown in phytohemagglutinin (PHA)-stimulated peripheral blood mononuclear cells, which rely largely on CCR5 for entry into monocytic cells. Two gp120 vaccines produced in mammalian cells have been evaluated in phase II trials. These vaccines are now being modified to include additional components derived from Thai or U.S. isolates; these vaccines will be evaluated to determine whether they induce antibodies that block infection via CCR5.

Preclinical research is attempting to increase the quality and breadth of neutralizing antibodies by selecting Env proteins from primary isolates of HIV as the vaccine antigen and by creating forms that may better reflect the native structure of the Env protein in infectious HIV.

In addition to subunit proteins, peptide vaccine approaches are also under investigation. Peptides can be produced by chemical synthesis. Branched-chain peptides, peptides that include both T cell and B cell epitopes, and peptides conjugated to lipids to stimulate cytotoxic lymphocytes are in evaluation. The goal of these approaches is to increase immunogenicity.

Live-Attenuated Vaccines

Historically, live-attenuated vaccines have been among the most efficacious viral vaccines. These vaccines use live virus that has been modified (attenuated) to make the virus less virulent. They induce both humoral and cell-mediated immunity and generally require only one or two immunizations, since the immune responses induced by live-attenuated vaccines are very durable.

Because of safety considerations, live-attenuated HIV vaccines have not been tested in humans. Safety issues include the possibility that such a vaccine could cause AIDS, at least in some individuals; that attenuated virus in the vaccine could revert to the wild-type, disease-causing virus; or that long-term infection could cause autoimmune or malignant disease. This concern is underscored by observations that small deletions in HIV accessory genes, those used to attenuate the virus, can be "repaired" after *in vivo* infection.

Live-attenuated AIDS vaccine studies have, however, been conducted using SIV in macaques. These SIV studies have shown that both a naturally occurring attenuated virus with multiple mutations and one created by deletion of one or more genes can serve as vaccines. Protective efficacy appears to vary inversely with the level of attenuation, increases over time after vaccination, and is strongest when the challenge virus is closely related to the vaccine. (Challenge virus is used in animal studies after vaccination to determine whether protection occurred.) One of these vaccines, in high dose, has caused death in neonatal macaques, and some individual juvenile or adult macaques may be susceptible to disease from these vaccines.

Research is ongoing to determine the mechanisms responsible for protection with live-attenuated HIV vaccines and to find safer ways to mimic the immune responses.

Particle or Whole-Inactivated Vaccines

Whole-inactivated viral vaccines are prepared by rendering the pathogenic virus unable to replicate, usually by a chemical treatment. These "killed vaccines" are potentially safer than live-attenuated vaccines; however, whole-killed vaccines often lose the potent, long-lasting immunogenicity of the live virus because the treatment used to inactivate the virus often destroys or alters important protective antigens. Incomplete inactivation could result in infection of vaccinated individuals by residual pathogenic virus.

To overcome some of these obstacles, researchers have produced virus-like particle (VLP) or "pseudovirion" experimental AIDS vaccines by recombinant technology. They express all or a portion of one or more structural genes of HIV or SIV and mimic the native expression of the particular viral protein(s). However, the pseudovirions do not contain the HIV genome, so they cannot produce progeny virus. The manufacture of these VLPs is complex, and given the genetic variability of HIV, these vaccines may need to include gene products from multiple strains of HIV.

To date, whole-killed HIV vaccines have not protected immunized chimpanzees against infection with the virus. Although macaques were protected against SIV challenge when immunized with inactivated SIV, the protection was due to antibodies to human cell line xenoantigens present in the vaccine preparations and challenge stocks. Macaques that were immunized and challenged with SIV grown in monkey cells were not protected from infection.

To date, only one VLP experimental AIDS vaccine (p17/p24:TY) has been tested in prophylactic phase I trials. This particle contains only a portion of core, without envelope, so it is more like a subunit than a complete HIV particle. Low levels of HIV-specific binding antibodies and T cell memory responses were generated in most of the volunteers after three or four immunizations. However, little HIV gag-specific CTL activity has been observed to date. In an ongoing study, volunteers receive intramuscular immunizations followed by boosts of the VLP orally or rectally to determine whether HIV-specific mucosal antibody responses are induced. Other recombinant particle vaccine candidates are in development or in the planning stages.

Recombinant Live-Vector Vaccines

Recombinant live-vector vaccines represent a novel vaccine strategy currently under development for HIV. These vaccines are produced by engineering viral or bacterial genomes to express the desired HIV antigen(s). Viral vectors can be constructed to contain one or more viral genes that cause infected cells to make the coded protein in native form. Recombinant viral vectors enter cells and allow the HIV or SIV proteins to be generated inside the cells; these proteins are then presented to the immune system in the same way that proteins from a virus-infected cell would be. As a result, vector-based vaccines induce both humoral and cellular immune responses. Importantly, immune responses can be generated to the vector as well as to the incorporated antigens. The immune responses to the vector could limit the effectiveness of subsequent immunizations using the same vector. When given in combination with recombinant subunit products, live-vector experimental vaccines have been shown to prime the recipient for augmented immune responses.

Live, infectious viral or bacterial vectors, genetically engineered to express genes of HIV or SIV, are being evaluated in animal models for their potential to prevent infection by HIV, SIV, or SHIV. (SHIV is a genetically engineered hybrid that has an HIV envelope and an SIV core.) Poxvirus recombinants were the first to be evaluated in nonhuman primates. Vaccines based on vaccinia virus, modified vaccinia ankara, or NYVAC (two attenuated vaccinia strains) have protected nonhuman primates from SIV, HIV-2, or HIV-1 infection when they were given alone or followed by immunization with purified envelope protein to boost the antibody response (prime-boost protocol). The data are varied, however; some animals have been protected against infection, some only against disease, and some have not been protected at all.

The AVEG has conducted phase I clinical trials to evaluate recombinant vaccinia-HIV gp160 with and without boosting with one of six different candidate recombinant gp120 or rgp160 vaccines. By itself, the vaccinia-gp160 induced little antibody; however, one or two doses of it primed for both gp160-specific CTLs and anti-HIV neutralizing antibodies. The priming effect was strongest in those volunteers who had never been vaccinated against smallpox. A phase I trial of a recombinant vaccinia-HIV vaccine, incorporating Env, Gag, and Pol antigens and boosted by rgp120 candidate vaccine, is under way in the AVEG.

Vaccines based on the canarypox virus have also protected nonhuman primates from SIV, HIV-2, or HIV-1 infection when given alone or followed by a boost. These vaccines are considered safer than vaccinia vaccines since canarypox fails to replicate in mammalian cells. Recombinant canarypox-HIV vaccines have also been shown to induce both anti-HIV neutralizing antibodies and CTLs in humans, regardless of prior vaccination with vaccinia. Boosting or concomitant administration of rgp120 or rgp160 increases the production of HIV neutralizing antibodies and may induce HIV-specific antibodies. Three different types of canarypox-HIV gp160 and canarypox-HIV gp120 experimental vaccines are undergoing testing in phase I trials in France and in the United States. In addition, a phase II trial of recombinant canarypox-HIV gp120, alone or in combination with rgp120, is being jointly conducted by the AVEG and the HIVNET (HIV Vaccine Prevention Trials Network) in 420 uninfected (seronegative) volunteers, many recruited from populations at high risk of HIV infection.

Another live recombinant vector experimental vaccine that has shown protection in nonhuman primates is an adenovirus-HIV envelope vaccine. It generated neutralizing antibodies and anti-HIV CTLs when administered in a prime-boost regimen with HIV envelope protein. The prime-boost regimen protected all chimpanzees from HIV infection when they were challenged shortly after the last immunization. These chimpanzees were protected 1 year later even though they did not receive a boost and were challenged with a higher dose of the same challenge virus.

A variety of other vector-based approaches are also being developed for HIV vaccines, including recombinant poliovirus, mengovirus, Venezuelan equine encephalitis virus, herpes virus, Semliki Forest virus, influenza virus, salmonella, Bacille Calmette-Guérin (BCG), Shigella, and lactococcus. Poliovirus-SIV, BCG-SIV recombinants, and Semliki Forest virus-SIV recombinants have been tested in nonhuman primates. Although initial studies have not shown protection against infection in small numbers of monkeys, further studies are under way. Much of this work is being performed through grants awarded in NIAID's Innovation Grant Program, and testing of a recombinant salmonella-HIV gp120 candidate vaccine in a phase I trial is planned by the AVEG.

Additional research is needed in several areas regarding live recombinant vector AIDS vaccines. Increasing the level of expression of HIV proteins in the recombinant vector may improve immune responses; vectors containing genes from primary isolates and nonclade B isolates should be evaluated; the optimum route, dose, and schedule of administration need to be determined; and the best way(s) to combine recombinant vector vaccines with subunit, DNA, or other vector experimental vaccines to maximize or optimize the immune response need to be determined.

DNA Vaccines

In DNA immunization, the host is immunized by direct administration of viral genes; the genes are composed of DNA that encodes for the antigen that would normally be produced by the cell infected with the virus. Several experimental DNA vaccines for HIV/AIDS have been produced and tested in small animals and nonhuman primates. In general, the results of these studies have been promising. DNA vaccines delivered intramuscularly or by gene gun have been shown to induce both neutralizing antibodies and CTL responses against HIV and SIV antigens. In one study, DNA immunization induced neutralizing antibodies and a vigorous CTL response; however, this immunization did not protect rhesus macaques from infection or disease on subsequent challenge with a pathogenic SIV after peak CTL and neutralizing antibody titers had waned. DNA immunization was, however, successful in protecting chimpanzees against a nonpathogenic HIV infection and in protecting rhesus macaques against a nonpathogenic chimeric virus (SHIV). In the latter study, the animals were first immunized with a DNA vaccine expressing HIV-1 IIIB Env and later received a booster immunization with a subunit protein vaccine composed of HIV-1 envelope protein prior to challenge.

Key issues for DNA vaccine development include optimizing antigen expression by DNA vaccine plasmids and optimizing immune responses to DNA vaccines in nonhuman primates and in human subjects. The development of new expression systems with more potent promoters, immunomodulatory cytokine genes, or costimulatory molecules, as well as formulation of DNA vaccines with adjuvants, cytokines, or novel delivery systems, is also being evaluated as ways to enhance the immunogenicity of DNA vaccines. Research is also needed to determine the most effective routes of administration and the kinetics of immunization and to evaluate the utility of sequential immunization (prime-boost) strategies using DNA as the primary immunogen followed by secondary immunization with subunit protein or vector-based vaccines.

A phase I clinical trial of two DNA candidate vaccines, one containing an HIV-1 Env and rev and the other a Gag-Pol construct, is under way through NIAID's intramural program and the AVEG. Additional trials are being planned.

NIAID remains committed to the discovery and development of a safe and effective vaccine for the prevention of HIV infection or disease. Toward that end, NIAID will continue to pursue a comprehensive range of basic, preclinical, and clinical research and will continue to evaluate these and other vaccine strategies and products as they become available.

Selected Topics in Vaccine Research and Development

MALARIA VACCINE DEVELOPMENT

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Introduction and Background: The Burden of Malaria

Once considered under control, malaria in recent years has reemerged as an ominous and global public health threat of monumental proportions. Today over 40 percent of the world's population lives in areas at risk for malaria. Some 300 to 500 million persons are infected. Children—especially those under the age of 5—and pregnant women—especially those pregnant for the first time—are particularly susceptible to the severe consequences of malaria. Each year 1.5 to 2.7 million people—mostly young children in sub-Saharan Africa—die of malaria.

The mortality attributed to malaria in endemic areas, however, tells an incomplete story. Malaria is often cited as a substantial impediment to economic and social development. Its effects are manifold but include decreased worker productivity, increased school absenteeism, and inaccessibility of natural resources. The chronic debilitation produced by malaria may also result in increased susceptibility to other infectious agents, either directly or indirectly. Supporting evidence for the magnitude of this effect comes from recent clinical trials in which childhood deaths from all causes were dramatically reduced by 15 to 33 percent following implementation of bed-net programs to reduce malaria transmission. Furthermore, in Africa, where malaria-infected individuals often develop life-threatening anemia, transfusion with unscreened blood has the potential to contribute to the spread of HIV / AIDS.

Malaria is flourishing anew for a variety of reasons. Vector control, long considered the mainstay of malaria control, has become increasingly problematic due to the spread of insecticide-resistant strains of mosquitoes. Resistance to commonly used antimalarial drugs such as chloroquine is now widespread, and malaria parasites appear to be capable of developing resistance to new drugs at an increasingly rapid rate. Recent experience has shown that as new drugs are introduced, costs and toxicities often limit their utility. Unfortunately, as the number of effective antimalarials shrinks, there are few promising new leads in the pipeline. Finally, economic development itself has contributed to the rise in the number of malaria cases as humans alter fragile ecological balances and come into increasing contact with mosquito vectors.

Malaria has demonstrated its capability to spread internationally at a rapid rate, and international travel and commerce have promoted its geographic dispersion. In recent years there have been more than 1,200 cases of imported malaria reported annually in the United States. Many of these have occurred in recent immigrants from endemic areas; since 1987, however, the majority of cases in civilians have occurred in returning travelers. Malaria has also been reported with increasing frequency of late in U.S. military personnel deployed in endemic areas (e.g., Somalia, Korea, Haiti). Although nowhere near the levels reported in the United States through the 1940s, malaria transmission still occurs sporadically in this country, and the potential exists for increasing incidence here due to the presence of competent mosquito vectors.

Because of malaria's renewed spread and growing global burden, its control is essential. Conventional control measures, however, appear increasingly inadequate to the task: As a result of the spread of drug-resistant parasites and insecticide-resistant mosquitoes, fewer tools to control malaria exist today than did 25 years ago. Moreover, since malaria afflicts many of the poorer nations of the world, any effective intervention must be cost-effective and (preferably) inexpensive. Historically, vaccines are widely believed to be the most cost-effective means of control. Successful vaccines for malaria could, it is widely acknowledged, significantly reduce morbidity and mortality, especially among young children and women, and accelerate social, economic, and human development.

Issues in Malaria Vaccine Development

At present no highly efficacious malaria vaccines are available, but there are good reasons to believe that such vaccines are feasible. Protective immunity has been demonstrated in animal models. It has been shown that populations in endemic areas acquire immunity as a result of naturally occurring exposure and that serum from immune individuals can transfer protection to nonimmune individuals. In human volunteer studies, vaccination with attenuated malaria parasites has been shown to elicit effective immune protection against challenge with infective malaria parasites. Finally, a novel candidate vaccine (see below) has demonstrated statistically significant protection in human volunteers who subsequently were experimentally challenged with malaria parasites.

Vaccines based on live, attenuated malaria parasites are economically and technically impractical, and research on malaria vaccines, therefore, focuses on recombinant or synthetic subunit vaccines. An optimal vaccine would have the ability to elicit protective immunity that blocks infection as well as prevents pathology and blocks transmission of parasites, and would most likely be a combination vaccine comprising subunits from different parasite stages (see below).

Compared to vaccines for most acute bacterial or viral diseases, development of malaria vaccines presents formidable obstacles, in terms of parasite biology, host immune responses, and both preclinical and clinical evaluation. Four species of protozoan parasites cause malaria in humans: *Plasmodium falciparum*, *P. vivax*, *P.*

malariae, and *P. ovale*. *P. falciparum* is responsible for the most severe form, including cerebral malaria. Because of its associated mortality, most vaccine efforts have been directed to *P. falciparum*.

Efforts to develop malaria vaccines, even for a single species of Plasmodium, must address a multiplicity of issues (see Table 1). First, which parasite antigens should be incorporated into a candidate vaccine? Malaria parasites have complex life cycles and antigenically distinct developmental stages, and each developmental stage may have multiple antigens that could serve as targets of an immune response. To make matters worse, each antigen may contain multiple epitopes and may exhibit variation within those epitopes.

Table 1.

Problems of Multiplicity in Malaria Vaccines

Multiple parasite species
Multiple antigens; multiple epitopes
Multiple antigenic variants
Multiple parasite developmental stages
Multiple vectors
Multiple epidemiologies
Multiple immune responses
Multiple objectives
Multiple endpoints

Host immunity to malaria is likewise complex. Naturally occurring immunity to malaria is slow to develop and wanes rapidly. Protective immunity appears to involve multiple different immune responses, both humoral and cellular. There is evidence, too, that malaria infection elicits immune responses that have been shown to correlate with adverse outcomes, suggesting that they contribute to the pathogenesis of disease.

Preclinical evaluation is complicated by the absence of well-characterized and validated laboratory model systems that reliably mimic or predict human immune responses. An *in vitro* culture system exists only for *P. falciparum*, and available animal models are inadequate to assess potentially prophylactic and therapeutic vaccines or to study mechanisms of pathogenesis. At present there are also no validated, reliable *in vitro* assays predictive of protective immunity in humans.

Finally, in clinical trials, how should one assess the efficacy of a given candidate vaccine? At present there are no diagnostic tests for malaria that have been shown to be uniformly rapid, inexpensive, sensitive, specific, and reliable in a field setting, although the need for standardized methodologies has been recognized. Also, further work needs to be done to define protection (e.g., absence of parasitemia vs. absence of pathologic complications vs. reduction of transmission) in a manner appropriate for the conduct of clinical trials. Although several field trials of an initially promising synthetic peptide-based malaria vaccine (SPf66) have been carried out in South America, Africa, and Thailand, the reported efficacies have differed substantially, perhaps in part due to differences in the definition of "efficacy" as well as epidemiology. Certainly, however, the reported levels of vaccine efficacy have not been demonstrated with confidence to be adequate to warrant widespread use of these SPf66 formulations.

Given that the ultimate malaria vaccine is likely to be a combination vaccine, what are interim vaccines likely to look like? In recent years two types of vaccines have been singled out for further discussion, namely, vaccines designed to prevent infection in travelers journeying to malaria endemic areas for recreation, business, or other purposes; or vaccines to prevent (severe) disease in populations residing in endemic areas. Whereas the former might be reasonably based on immune-mediated elimination of the infecting pre-erythrocytic parasite stages, the latter would be based on asexual erythrocytic stages responsible for producing the major pathologic outcomes associated with malaria. A third type of vaccine, based on sexual stages of the parasite, would prevent transmission and development of parasites in the mosquito vector. As such, it is considered an "altruistic" vaccine since it would not directly prevent the infected individual from becoming ill but would prevent malaria from spreading to other individuals.

A further question is, what type of approach should be taken with regard to vaccine development? Broadly speaking, approaches can be divided into empirical or rational ones; the former simply requires assays for safety and protection from infection in the vaccinated individual, whereas the latter requires in addition an understanding of underlying mechanisms of protection and pathology. It should be emphasized that these approaches are not mutually exclusive and that rational approaches have at their core empirical observations. Nevertheless, most vaccines to date have been developed largely based on empirical approaches. The range and complexity of issues involved in malaria vaccine development, however, require that efforts be made to establish an integrated, rational basis for future efforts.

Addressing the Scientific Challenges

NIAID has had a longstanding and growing commitment to malaria vaccine research. In FY 1996 alone, NIAID spent over $19 million on malaria research, roughly a quarter of the total amount of money spent globally on malaria research. The current portfolio is predominantly (>85 percent) composed of unsolicited research awards and strongly emphasizes laboratory-based basic research. Clinical and field-based research is primarily supported through two special programs, the International Collaborations in Infectious Diseases Research and the Tropical Medicine Research Centers. In addition, NIAID is currently supporting clinical trials of two new antimalarial therapies in Ghana and Thailand, and, through the Vaccine and Treatment Evaluation Unit network, phase I/II trials of promising vaccine candidates.

An important recent development has been the recognition that malaria research, including vaccine research, needs to be conducted in an international context. One approach is embodied in the Multilateral Initiative on Malaria (MIM), which calls on research and public health agencies internationally to join forces to combat malaria. In January 1997, at an initial international meeting in Dakar, Senegal, of scientists, administrators, and public health officials, a series of high-priority research topics were identified. (The report of the Dakar meeting is available on the NIAID home page, http://www.niaid.nih.gov.) At followup meetings in The Hague, Netherlands, and London, U.K., research agencies discussed ways in which they could support these efforts, and a task force was

formed to address the need for research capacity strengthening on malaria in Africa. Within the evolving context of the MIM, multinational malaria vaccine research is clearly a priority.

One particularly striking example of current multilateral collaboration is the Malaria Genome Sequencing Project, a large-scale effort to sequence the *Plasmodium falciparum* genome, which is supported by NIAID, the Office of the Director of the National Institutes of Health, the Burroughs Wellcome Fund, the Wellcome Trust, the U.S. Department of Defense, and the Institute for Genome Research. It is expected that the availability of the *P. falciparum* genome sequence will facilitate the identification of new targets at the molecular level for intervention and control, including antigens for inclusion in candidate malaria vaccines.

Recent advances in malaria research are also allowing a more focused approach to vaccine development. For example, stable transfection of *Plasmodium* parasites has recently been achieved. By studying knockout mutants of malaria parasites, NIAID-supported investigators at New York University School of Medicine have recently documented important biological functions for two leading vaccine candidate antigens, CSP and TRAP. Such studies now provide a rational basis for understanding the mechanisms of protective immunity directed against these antigens.

Another significant recent finding was reported earlier this year by investigators at the Walter Reed Army Institute of Research and their collaborators at SmithKline Beecham. These investigators were able for the first time to elicit with a synthetic peptide-based vaccine significant sterilizing protective immunity against experimental challenge in human volunteers. Plans for further evaluation of this candidate vaccine are now under way.

Today there are many scientific opportunities in malaria vaccine research and development that make it an attractive research and public health investment (shown in Table 2). Taking advantage of these opportunities, however, is hindered by a number of gaps in research and development. Furthermore, one clear lesson from the clinical trials conducted to date is that a defined preclinical and clinical development path for malaria vaccines is lacking. To address these issues, NIAID has developed a plan for structuring its ongoing commitment to malaria vaccine research and development, the principal elements of which are shown in Table 3. This plan, which draws on expertise present in intramural and extramural NIAID programs and extramural institutions, should allow for a coordinated, internally consistent expansion of NIAID-supported research to allow accelerated identification, preclinical development, and clinical evaluation of promising candidate malaria vaccines. A detailed description of the plan, which was endorsed by a Blue Ribbon Panel of extramural scientists in June 1997, is available on the NIAID home page (URL: http://www.niaid.nih.gov). Efforts are already under way to implement some of the major goals of the plan, including establishing a repository of well-characterized reagents to improve access to malaria research materials for malaria investigators; expanding parasite genome sequencing efforts to include not only *P. falciparum* but also *P. vivax*, another major malaria parasite of humans, and an appropriate rodent malaria that provides a model system for functional analysis of gene products *in vivo* and evaluation of protective immunity; and expanding current malaria vaccine production and evaluation efforts through collaborations between intramural and extramural scientists.

Table 3.

Highlights of the NIAID Malaria Vaccine Research and Development Plan 1997

Improved access to research materials
Discovery and preclinical testing of new vaccine candidates
Production and evaluation of candidate malaria vaccines
Clinical research and trial preparation sites in endemic areas

Table 2.

Malaria Vaccine Research and Development

Opportunities	Gaps
Vaccines can be targeted to different stages in the life cycle to prevent infection, disease, or transmission	Not known which vaccines will be appropriate for different target populations
Many candidate vaccine antigens already exist	Little interest from industry, and a preclinical development program is not available
Protective efficacy of candidate vaccines can be assessed in nonhuman primates	Limited access to primate testing
Detailed studies of immune responses to vaccines possible in the United States	Responses in naïve subjects may not be predictive of those in populations residing in endemic areas; field-based research on immunity and pathogenesis is currently insufficient
Challenge trials of pre-erythrocytic vaccines can take place now in the United States	Trials in endemic areas will be needed, and infrastructure to conduct clinical research and trial programs needs to be strengthened

Summary and Conclusions

The reemergence of malaria as a growing public health threat of international proportions, coupled with unprecedented scientific opportunities in the field, mandates that renewed and expanded attention be focused on the research and development of malaria vaccines. To do so effectively, however, requires that malaria research be expanded in an international context and that judicious and visionary deployment of resources occur in a sustained manner. NIAID, through collaborations with its research partners and implementation of its plan for malaria vaccine research, is moving to create both the research basis and infrastructure to assure that accelerated development of malaria vaccines occurs.

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TUBERCULOSIS VACCINE DEVELOPMENT

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Introduction

Mycobacterium tuberculosis, the bacterium that causes tuberculosis (TB), infects one in every three people on Earth, approximately 2 billion people, and last year killed an estimated 2 to 3 million individuals, more than any other single infectious agent. This devastating burden of disease continues despite the World Health Organization (WHO) Global TB Programme's significant efforts to control TB in developing countries through Directly Observed Treatment Short-course (DOTS) and worldwide immunization of neonates by the WHO Expanded Programme of Immunization with BCG (Bacille Calmette-Guérin), a live, attenuated anti-TB vaccine.

M. tuberculosis-infected, immunocompetent individuals have an estimated 10 percent lifetime risk of contracting active TB disease. The majority will remain latently infected, not demonstrating any active symptoms of disease, but exhibiting a positive delayed type hypersensitivity reaction on skin test challenge with PPD (Mantoux skin test). HIV-infected and other immunocompromised individuals, however, bear a much higher risk of developing active TB disease—estimated at 8 to 10 percent per year. Magnifying the severity of the global situation is the fact that HIV and *M. tuberculosis* infections are synergistic. *M. tuberculosis* can increase the replication rate of HIV, and coinfection with *M. tuberculosis* speeds the progression and severity of AIDS in HIV-infected individuals. In 1996, TB became the leading cause of death in HIV-infected individuals worldwide, responsible for approximately one-third of all AIDS deaths. Conversely, HIV infection may increase susceptibility to primary infection and dramatically increases the risk of *M. tuberculosis*-infected individuals of developing active tuberculosis. In parts of Africa and Asia, TB case rates are climbing rapidly as the HIV epidemic spreads. The chairman of the Kenya Association of Physicians reported that the number of TB cases in Kenya increased tenfold in the past decade.

The TB situation is serious in the United States and other developed countries, as well as in the developing world. Improved control has been achieved overall in the United States since the resurgence of TB in the late 1980s and early 1990s—case rates for the country as a whole have decreased for the past 4 years. Nonetheless, in 1996, the Centers for Disease Control and Prevention (CDC) reported 21,337 cases of TB in the United States, and up to 15 million people in this country are currently believed to be infected with *M. tuberculosis*. Case rates remained level or increased in 23 States and the District of Columbia in 1996 compared to 1995. Approximately one-third of U.S. TB cases are now found in the foreign-born, and this percentage is increasing.

Multiple drug-resistant TB (MDR-TB) is a significant concern in the United States as well as in the rest of the world. The CDC reported in September 1997 that while overall MDR-TB currently represents 2.2 percent of all TB in the United States, a strain of MDR-TB that 6 years ago was in only 13 States has now spread to a total of 42 States. MDR-TB is both difficult and extremely costly to treat, and because treatment is often relatively ineffective, patients remain infectious much longer than do those receiving proper treatment for drug-susceptible TB. Moreover, as with other forms of TB, MDR-TB can be spread by casual contact. The WHO reports MDR-TB to be as high as 14 percent in some parts of eastern Europe. The imperative to control TB better is increased by the knowledge that drug-resistant tuberculosis is created almost entirely by delayed diagnosis and improper or inadequate therapy. Dr. James Musser and colleagues reported that greater than 95 percent of gene variation in *M. tuberculosis* is caused directly by human use of antibiotics.

Multiple factors have coalesced to render development of improved anti-TB vaccines a necessity for adequate control and elimination of tuberculosis in the United States and worldwide. These include the spread of MDR-TB, the global burden of the TB epidemic and the growing TB / HIV co-epidemic in large regions of the world, the enormous practical barriers to controlling TB adequately through administration of what is a complicated and costly treatment regimen, inadequate diagnostic methods, and the relative inefficacy of the current BCG vaccines.

BCG Vaccines

In 1996, 100 million infants were immunized with BCG; since 1921, more than 3 billion doses of BCG have been administered worldwide. BCG was developed at the Institut Pasteur in 1906–1919 by serial passage of a mixed culture of *M. bovis*, and was first used in humans in 1921. BCG is one of the six basic antigens of the Expanded Programme of Immunization, and Holland and the United States are the only two countries that do not recommend universal BCG vaccination of children.

A review of the literature, including meta-analyses of BCG vaccination studies, demonstrates varying efficacy for BCG in providing protection against tuberculosis. Against miliary TB and TB meningitis in children, BCG's reported efficacy ranges from 46 to 100 percent. However, against pulmonary TB, which represents the majority of the burden of this disease, BCG's efficacy ranges from 0 to 80 percent.

Numerous factors have been cited as possible explanations for this variability, including methodological differences among the studies, variations among BCG strains, differences in the strains of *M. tuberculosis* in different parts of the world, nutritional factors, environmental factors, varying exposure to environmental, nonpathogenic mycobacteria, and genetic differences in host popula-

tions. Most likely, a combination of factors is responsible for the variable efficacies observed, with BCG strain differences and varying exposure to environmental mycobacteria perhaps playing the strongest roles.

A recent report presents evidence that efficacy of any given strain of BCG has decreased as passage number increased, while induced delayed-type hypersensitivity (DTH) response has remained high. Historically, manufacturers have selected for strong DTH response while trying to decrease reactogenicity/toxicity of BCGs, believing that DTH response correlated with protective immune response.

More recent evidence suggests that DTH and protective immune responses are not necessarily intertwined. While raising an important point about current BCGs, the report does not address available data showing high efficacy of a single BCG preparation in one part of the world, while providing little or no protection in another. Therefore, while probably a contributing factor, attenuation with serial passage and selection for decreased reactogenicity cannot represent the sole explanation for the variability in protection against pulmonary TB seen in different studies.

Efficacy of BCG vaccination appears to vary with geographic latitude—the farther from the equator, the more efficacious the vaccine. Environmental mycobacteria are more prevalent closer to the equator. Presumably, exposure to nonpathogenic mycobacteria induces a degree of protective immunity in the exposed human populations, thereby rendering less evident any potential protection from BCG.

In addition to providing relative protection against extrapulmonary TB in children, BCGs have demonstrated efficacy against leprosy, another mycobacterial disease. As a result, it is difficult to imagine simply replacing BCG with a potentially better anti-TB vaccine, especially in the context of clinical trials. Rather, in most parts of the world, new, hopefully more efficacious, anti-TB vaccines will have to be tested and administered in addition or subsequent to BCG vaccination.

Status of TB Vaccine Development

As BCG vaccination is unlikely to have significant impact on the TB epidemic, development of improved anti-TB vaccines is a high priority for NIAID and the TB research community. In the past few years, numerous laboratories have undertaken to develop potential vaccine candidates, following a range of strategies. These approaches include development of modified BCGs, live-attenuated strains of *M. tuberculosis*, subunit vaccines, and naked DNA vaccines. Literally dozens of candidates representing all these strategies have been developed in academic and industrial laboratories in the past 3 to 5 years.

Evaluation of these candidates and prioritization for human clinical trials must rest on studies in animal models. The major animal models of tuberculosis currently available are based on the mouse, guinea pig, rabbit, and more recently the cynomolgus monkey. NIAID has established a contract-supported service for testing vaccine candidates in the mouse, guinea pig, and rabbit aerosol challenge models.

Each of the available models is considered to have different potential advantages and disadvantages. The mouse model is relatively inexpensive and can take advantage of well-defined inbred mouse lines, a comparatively well-defined immune system, and the availability of immunologic reagents. However, the mouse is relatively resistant to tuberculosis, unlike humans, and does not demonstrate the caseating necrosis and liquefaction that are hallmarks of human tuberculous pathology.

The guinea pig model is somewhat more expensive, has only very limited availability of inbred lines, and is less well understood immunologically, but, like humans, is exquisitely sensitive to aerosol infection by *M. tuberculosis*. The rabbit model, pioneered by Lurie and championed by Dannenberg, is costly, with little available genetic or immunologic infrastructure, but most closely mimics human pathology by demonstrating caseating necrosis and liquefaction subsequent to infection with virulent *M. tuberculosis*.

The more recently developed cynomolgus monkey model shows promise for providing a model of latent infection similar to that established by most humans infected with *M. tuberculosis*. Unfortunately, it remains unclear which, if any, of these models adequately predicts the human protective immune response. This uncertainty represents a major limitation in the ability to determine whether or when a candidate is ready to enter human trials.

Modified BCGs

Recombinant DNA methodologies have been used to add expression of putative protective antigen(s) or cytokine(s) to BCG to boost its protective effect. This approach takes advantage of BCG's ability to persist intracellularly in the host, mimicking *M. tuberculosis* infection, and its endogenous adjuvanticity. To date, several candidates of this type have shown protective efficacy in mouse and/or guinea pig similar to but not significantly better than BCG.

BCG has also been modified through the creation of auxotrophic mutants. Currently, in some countries where BCG is otherwise administered universally, it is recommended that HIV-positive individuals not be BCG vaccinated, due to the fear of causing disseminated BCGosis in these immunocompromised hosts. In mice, some auxotrophic BCG strains grew, induced an appropriate T cell response, and were ultimately cleared, even by SCID (severe combined immunodeficiency) mice. Auxotrophic strains of *M. tuberculosis*, in contrast, killed infected SCID mice, indicating that further attenuation of *M. tuberculosis* would be necessary before such vaccine candidates could be tested in immunocompromised humans. However, it was not demonstrated in the reported experiments whether revertants had grown out and killed the mouse hosts or whether *M. tuberculosis* differs from BCG in its ability once inside an animal host to acquire the necessary amino acids for survival.

Attenuated Strains of *M. tuberculosis*

Sequencing of the entire *M. tuberculosis* genome—both a laboratory strain (H37Rv) and a highly transmissible, clinical isolate (CSU 93)—will be completed in the next few months. It is hoped that this information, combined with recently developed techniques for *M. tuberculosis* mutagenesis and allelic exchange, and functional analyses in animal models will enable relatively rapid advances in the identification of *M. tuberculosis* virulence determinants. Such knowledge would make it possible to rationally attenuate *M. tuberculosis* as the basis for vaccine candidates.

Another approach, already under way, to identifying virulence factors is the determination of genetic differences between BCG and a virulent strain of *M. bovis*, using subtractive hybridization. These experiments identified three regions of difference between BCG and virulent *M. bovis*. One of these difference regions, termed RD1, was absent from all BCGs tested but present in all clinical isolates of *M. bovis* and *M. tuberculosis* examined. More detailed examination of RD1 may reveal a gene or genes whose functional deletion would attenuate *M. tuberculosis*. Alternatively, one can imagine creating and testing a recombinant BCG expressing a subset of the RD1 sequences, with the hope that such a construct might induce stronger protective immunity than BCG itself, while retaining the avirulent nature of BCG. Work in this area should progress rapidly over the next couple of years.

Subunit Vaccines

Candidate subunit vaccines, consisting of only a subset of *M. tuberculosis* antigens rather than the whole bacterium, that could induce a strong protective immune response would be attractive because of their perceived relative safety and potential ease of manufacture. Several investigators are trying to develop TB vaccine candidates of this type, based on a variety of "culture filtrate proteins" (CFP)—i.e., mycobacterial protein antigens visible to the T cell immune machinery.

While some investigators are testing the ability of complex CFP preparations containing many proteins to induce a protective immune response in mice and guinea pigs, others are pursuing individual, purified proteins or defined mixtures of a relatively small number of purified proteins. To date, some of the most promising results have been seen in mouse and guinea pig aerosol challenge experiments following vaccination with a preparation of *M. tuberculosis* culture filtrate proteins combined with MPL adjuvant and cytokines interleukin (IL)-2 and IL-12. This combination provided protection at least equivalent to that of BCG in these animal models.

Rather than basing vaccine candidates on whole protein preparations, some investigators are trying to identify peptide antigens that could evoke a protective immune response. Current data suggest that combinations of several such peptides will be more effective than any individual peptide. Prediction of major histocompatibility complex (MHC)-binding epitopes is now possible through computer algorithms, and efforts are under way for annotating the completed *M. tuberculosis* genomic sequence using this type of analysis.

These studies provide an obvious example of the potential for rapid advances made possible by whole genome sequencing of a microbial pathogen. Similarly, investigators at Colorado State University are defining the precise makeup of mycobacterial CFP preparations by 2-D gel analysis linked to mass spectrometric N-terminal peptide sequencing. These relatively easily obtained partial sequences will then be matched to the complete genome sequence to discern rapidly the identity of each protein in the CFP preparation.

Few nonprotein antigens are currently under investigation as the basis of potential anti-TB vaccines. Exceptions include mycobacterial cell wall mycolic acids and carbohydrate moieties, which are being investigated for antigenicity and the ability to induce protection. More extensive work in this area is warranted.

DNA Vaccines

A major advance in vaccinology has been the relatively recent recognition that immunization with DNA encoding appropriate antigens can confer protection against a variety of bacterial, viral, and parasitic pathogens. The potential efficacy of DNA vaccination against tuberculosis was first demonstrated by Silva and Lowrie, who showed that immunization of mice with the J774 macrophage cell line transfected with the *M. leprae* hsp60 gene provided protection against intravenous challenge with virulent *M. tuberculosis* and BCG. Direct DNA vaccination of mice, using DNA encoding the major secreted protein, Ag85A, hsp65, or the 36 kDa proline-rich mycobacterial antigen, has also been accomplished and demonstrated to provide some protection against challenge with *M. tuberculosis*.

DNA vaccination offers several advantages, including enhanced safety and relative ease and low cost of manufacture. As a result, various approaches are being explored for increasing expression of the encoded antigens, and thereby, it is hoped, increasing protective efficacy. These approaches include comparing expression vectors containing different signal sequences, altering codons to optimize codon usage, and mutagenizing potential glycosylation sites. It is speculated that N-linked glycosylation of these bacterial antigens may interfere with their normal processing and presentation to the host immune system. Ag85A constructs are being tested in the guinea pig long-term challenge model under an NIAID contract. Attempts are also being made to enhance their immunogenicity by coexpressing cytokines and/or altering the adjuvant(s) used.

"Whole genome" approaches to identifying mycobacterial protective antigens are also under way. One technique, pioneered by Stephen Johnston and known as "expression library immunization," attempts to screen all potential open reading frames in an animal challenge model for those that encode protective antigens. Such antigens, once identified, could form the basis of either a protein subunit vaccine or a DNA-based vaccine.

Nonpathogenic Mycobacteria

Several nonpathogenic mycobacteria have been proposed as potential tuberculosis vaccines, including *M. habana*, *M. microti*, and *M. vaccae*. Most recently, *M. vaccae* has been evaluated as an immunotherapeutic agent in several trials, including a recently completed phase III trial in Durban, South Africa, and an NIAID-supported phase I/II trial in Kampala, Uganda, designed to look at early bactericidal activity in addition to time to sputum conversion. To date, the preponderance of evidence suggests that *M. vaccae* is not an effective immunotherapeutic for tuberculosis, at least when administered in a single dose, in addition to standard antituberculous drug therapy.

Microbial Vectors

M. vaccae and *M. smegmatis* are also being used in vaccine candidates as vectors to overexpress mycobacterial antigens, including the 19 kDa, Ag85, and 45 kDa proteins. Others are exploring the potential of using nonmycobacterial microbes, such as *Salmonella* and *Vaccinia*, as vectors for overexpressing mycobacterial antigens.

Challenges to Improved TB Vaccine Development

A number of significant challenges face those interested in developing improved TB vaccines. Many of these challenges derive from issues of clinical trial design, such as defining what a vaccine candidate could accomplish: inhibit initial infection, block transmission, prevent primary disease, or block reactivation of latent infection? A vaccine designed for delivery to teenagers or young adults who are post-BCG vaccination and/or exposure to *M. tuberculosis* could be tested in endemic areas in a much shorter timeframe than a vaccine meant to replace BCG for neonatal delivery (potentially requiring decades of followup to assess efficacy). The available animal models must be modified to test the desired purpose of candidates. Models of latent infection and reactivation are under active development.

Another unfulfilled need for effective clinical trials is the identification of correlates of the human protective immune response. This is an area of active investigation but with no satisfactory resolution to date. The need for correlates of protection also raises the already mentioned limitation that none of the available animal models necessarily replicates the human immune response. Animal models can therefore be used to provide preclinical evidence of safety and some indication of potential efficacy relative to BCG (for what this comparison is worth, given the limitations of BCG vaccination in humans discussed above), but any true indication of efficacy will depend on results of human trials.

The past few years have been a time of incredible productivity with respect to development in the laboratory of potential vaccine candidates and testing in short-term animal models. Active discussions are under way within the research and TB control community as to how best to design clinical trials of potential TB vaccines. The challenge for the next few years is to rationally prioritize the plethora of candidates for human clinical testing and design the necessary trials and infrastructure.

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UPDATE ON VACCINE SAFETY

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Vaccine safety continues to be highlighted in both the popular press and the medical literature and should be considered in an annual report on the status of vaccine research and development. The popular press has denounced the vaccine industry as vested in selling existing vaccines to a billion-dollar industry. Articles aimed at parents have discussed the pros and cons of having their child vaccinated, placing the onus of decisionmaking both on the individual as well as the public health level. Scientific publications continue to explore and wrestle with the complexities of establishing or refuting the causal association between vaccination and a variety of conditions, including the very disease the vaccine is supposed to prevent (polio), autoimmune diseases (Crohn's disease), and cancers. The power of anecdotal science, the questioning of "establishment" dictums, the uncertainty inherent whenever technology advances, and the lack of epidemiologic tools to prove something does not exist make this an important, challenging but extremely difficult arena.

The term "safety" has a variety of implicit meanings. In legal terms, it is the "relative freedom from harmful effect when a product is prudently administered, taking into consideration the character of the product in relation to the condition of the recipient at the time" (21 CFR 600.3 (p)). The assurance of vaccine safety is the result of a massive, unrecognized effort spanning research; development; laboratory and clinical testing of vaccines; production in specialized, stringently regulated facilities; careful evaluation of data prior to licensure, as well as vigilance postlicensure. From a public health perspective, it results from the comprehensive assessment of the risks and benefits of a vaccine in the context of risks of the disease and availability of other strategies for disease control. In the media, vaccine safety means sensationalized reports—often but not always erroneous—of the lack of safety.

In January 1997, a report from the Department of Health and Human Services' Task Force on Safer Childhood Vaccines affirmed that the stability of the immunization program continues to depend on public confidence in vaccines. The task force, which examined all aspects of vaccine safety, made recommendations to strengthen the U.S. vaccine safety system, spanning research, manufacture, education on the safe use of vaccines and appropriate knowledge of risks and benefits, and national systems to monitor for vaccine reactions. While responsibility for assurance of product safety rests with the Federal Government through the Food and Drug Administration's regulatory authority for biologics, for the immunization program "safety" requires participation from other Federal agencies, including the Centers for Disease Control and Prevention (CDC), the National Institutes of Health, Health Resources and Services Administration, and the Department of Defense. Ultimately, safety is achieved from the concerted efforts of scientists, doctors, nurses, parents, and manufacturers—indeed, every step of the pathway from production, storage, shipping, and effective and safe use of the vaccine. The National Vaccine Program Office (NVPO) will continue to coordinate this effort.

The paradox of immunization is that although vaccines must be as safe as possible to be licensed for use in healthy children to prevent disease, no vaccine or medicine is 100 percent safe. When a disease rages through a community, as poliovirus once spread in the summers, paralyzing children and leaving devastation and death in its wake, the new polio vaccines were perceived to be a precious gift from science. The day after the first clinical trial ended in 1955, the vaccine was licensed and parents lined up their children for vaccination. Forty years later, the scenario in the United States had changed. While there had been no poliomyelitis due to naturally occurring infection for 15 years, about six to eight cases occur every year due to the vaccine itself. This phenomenon, called vaccine-associated paralytic poliomyelitis (VAPP), is due to the potential for reversion to neurovirulence following immunization with the live-attenuated polio vaccine. Although this risk—about 1 per 1,000,000 recipients of oral polio vaccine and their susceptible contacts—may be perceived to be quite rare, it is now unacceptable to many, as the disease has been eliminated in the Americas and a safer alternative—the enhanced inactivated polio vaccine (eIPV)—exists. After intense public debate, in 1997 both the American Academy of Pediatrics and the CDC's Advisory Commission on Immunization Practices recommended a transition to increased use of a safer, inactivated polio vaccine (IPV).

The performance of the polio vaccine has not changed in the 40 years—rather, it is the perception of the balance of risks versus benefits that has altered. For many reasons, the polio policy decision represented a microcosm of the vaccine safety debate. Its implementation continues to raise a number of difficult questions:

- How to weigh potential benefits to individuals versus society, if these are in conflict?
- How much is society willing to pay for the safer vaccine to prevent these rare events?
- How to balance the benefits of the new recommendations to the U.S. population, compared to potential effects on the global eradication program, where too early a shift to IPV could slow eradication?
- Can the public or private systems for delivery of vaccines handle the increased complexity of the immunization schedule when an easily given oral vaccine is changed to an additional injection, without risking decreased immunization rates and outbreaks due to other vaccine-preventable diseases?

- Does the willingness to use the safer vaccine increase when children are mandated by State law to be vaccinated before school entry, thereby removing, in States without philosophical exemptions, parental choice?

Evaluation of risk associated with vaccines was the subject of intense study by a group under the auspices of the Institute of Medicine (IOM) of the National Research Council. Two indepth reviews of all available information on the safety of childhood vaccines were published in 1991 and 1994. The groups reviewed available case reports, papers, and reported safety problems. The reports documented that rare adverse events accepted to be due to vaccination, such as anaphylaxis following injected vaccines, do occur. The reviewers found that the existing data were sufficient to establish the causal relationship between vaccines and thrombocytopenia, vaccine-associated poliomyelitis, and death from measles vaccine-strain viral infections, as well as reject causation to infantile spasms or sudden infant death syndrome (SIDS).

There were many more conditions, however, for which the data are not clear-cut, or for which there are simply not very good data. It is this gray zone that is most troubling for many parents, who want to protect their children but are fearful of the risks. Here, science may reach its limits—at least thus far. Epidemiologic methods cannot prove that something does not exist, although large and intense studies can assure us that if it does exist, it is extremely rare, perhaps so rare as to not be measurable. This becomes even more difficult with extremely rare conditions (such as encephalopathy); when the lag time between the inciting event is years distant from the condition it is supposed to cause (asthma or diabetes); or when comparative studies are difficult to conduct because the vaccine is effective and very few individuals are not immunized.

A broad array of allergic, autoimmune, and neurologic conditions have been charged to be due to vaccination. Dispelling these fears is difficult but requires a robust and ongoing scientific effort to generate required laboratory, epidemiologic, and microbiologic data. For example, for many years SIDS was thought by some to be due to vaccination. Carefully conducted studies in populations failed to find an association with vaccines. Infants—the target age group for immunization because they are at high risk from the infections prevented by vaccines—receive vaccines at about the same age that developmental delays, other neurological symptoms, or SIDS is often diagnosed. This "coincidence" becomes difficult to untangle, and for parents of affected children, difficult to believe. Reassurance came more recently when the "Back to Sleep" position for sleeping infants lowered the mortality rate from SIDS in the United States.

There are other controversies that have been ongoing for a number of years without resolution. For example, in 1961, a cancer-causing virus of monkeys called SV40 was found to contaminate some of the oral polio vaccine grown in monkey kidney cells. Before it was recognized, millions of children were vaccinated with this vaccine. Tracking and followup of large populations in the United States and Sweden for more than 40 years have failed to find an increased incidence of cancer in these populations. New technologies (such as polymerase chain reaction) continue to be developed to detect any adventitious agents in the vaccine supply, and now the challenge becomes how to interpret the results and evaluate the implications.

The perception of risk becomes the driving force behind changes in the way vaccines are used by entire countries. The old, whole-cell vaccine available then to protect against diphtheria, tetanus, and pertussis (DTP) was blamed for many neurologic conditions based on reactions ranging from prolonged crying to seizures or coma. In the 1980s, concerns about pertussis vaccines resulted in a loss of confidence, dropping immunization rates, and national epidemics of pertussis disease in Great Britain and Sweden. The limited number of vaccine manufacturers threatened to further decrease in response to liability surrounding the DTP vaccine! After 15 years of enormous efforts by public health, scientific, and parent advocates and industry, three new acellular pertussis vaccines with an improved safety profile are now licensed in the United States. Other countries have chosen not to license these vaccines because of their higher costs. Whether any new vaccine causes extremely rare but serious illnesses can only be learned as the vaccine is used in millions of people and with ongoing surveillance.

However, the unstable vaccine supply posed a national health threat in the 1980s. In an atmosphere of urgency and compromise, medical, legal, and consumer groups worked with Congress to enact into law the National Childhood Vaccine Injury Act of 1986. As a result, the Federal Government instituted mandates for office record keeping, scientific studies of vaccine reactions, distribution of vaccine information for families and patients, and a national surveillance system to monitor adverse events. Liability was addressed with creation of the National Vaccine Injury Compensation Program, a no-fault approach to compensation through use of a table of compensable injuries. Litigation has now shifted to this simplified system, which provides a presumption of causation if a Vaccine Injury Table condition occurred in a prescribed timeframe, assuming certain legal requirements are met and another cause of the illness is not present. Recognizing the science is dynamic, the table has been updated twice based on the IOM reports. Operational since 1988, the program is designed to cover all routinely given vaccines and is financed by an excise tax on every dose of covered vaccine sold. As of July l997, more than 1,250 awards had been made to families or individuals. The program has streamlined the long, burdensome civil process and added more consistency to awards. While payments cannot adequately compensate for injury suffered, they do remove the financial burden for those conditions set forth in the table.

By most standards, the Federal system has met its goals of compensating individuals, stabilizing the marketplace (supply/pricing), and reducing health care provider and manufacturer liability. Only a small number of lawsuits are reportedly filed annually against U.S. manufacturers, and there is little evidence that those who are rejected by the Federal system or who chose to not accept compensation seek compensation elsewhere. Analysis of medical information in light of the IOM studies seems to show that most injuries and deaths are non-vaccine-related, caused by prenatal, metabolic, or other kinds of developmental conditions seen in children. If anything, this confirms the system of vaccine safety and the performance of products given routinely to healthy children and adults.

The benefit-to-risk balance of vaccination is not static. On one hand, diminished preventable disease is welcome but brings with it decreased awareness and a shift in the benefit/risk equation

toward concern. On the other hand, not giving vaccines will result in lost opportunities to eradicate disease (e.g., smallpox) and risk epidemics of the past.

Fortunately, the science of risk communication continues to advance, with the goal of learning what influences a person's view of risk, as well as what types of communication better inform and/or change behavior. Individual educational level, experiences, beliefs, attitudes, and values need to be considered, as well as perception of the risk of disease, the ability to control those risks, and the preference for one type of risk over the other. For some who choose alternative health approaches (e.g., holistic medicine), immunization may not be perceived as beneficial or they view the risk disproportionately. Others may dwell on sociopolitical issues, such as mandatory vaccination, informed consent, and individual rights versus societal welfare.

Today, vaccine risk information is transmitted through use of the CDC's Vaccine Information Statements, a relatively simple support tool to the informed consent process before vaccination. Other resources should be available for the more interested parent or vaccine recipient. Nothing can replace the value and trust placed on physicians and health care providers, who must both stay current with science and be prepared to address questions and concerns that are part of today's discerning, informative landscape. Not all patients will accept reassurance and choose to be immunized. Ultimately, it is trust—a fragile resource—that will continue to provide vaccine safety assurance as the last step in the long line of policies and procedures that make U.S. vaccines among the most safe and effective in the world.

The concerns of vaccine safety should be placed in context to the good news about immunization. Immunization rates in children are now the highest they have ever been in the United States, and the infectious diseases they prevent have reached new troughs. On a global level, the eradication of polio is being battled country by country. *The Jordan Report* is filled with fascinating scientific advances: promising vaccines against Lyme disease; genetically engineered potato or banana; and the intranasal live-attenuated influenza vaccine.

Progress continues in the face of efforts to balance the need to prevent additional infectious disease killers through development of new—and safe—vaccines.

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CANCER VACCINES

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Various immunotherapies administered to patients with advanced cancer have been shown to produce a small percentage of objective tumor responses (for example, with administration of interleukin-2 or monoclonal antibodies directed against tumor-associated antigens). Some of these responses have been complete and durable and have provided substantial benefit to individual patients. It may be possible to improve and expand upon the limited antitumor activity of immunotherapy by specifically and potently inducing immune responses against tumor antigens *in vivo*. Consequently, substantial efforts have been devoted to development of cancer vaccines. Rapid progress in development of cancer vaccines occurring over the past decade has been made possible by a vastly greater understanding of cancer biology and immunology and the substantial progress in molecular biology. These advances have resulted in the identification of many potential tumor antigens as well as novel techniques for more effective immunization.

Some key differences exist between vaccines for cancer and the more familiar vaccines developed for infectious diseases. In the field of infectious diseases, a vaccine is used to induce an immune response against a pathogen that will either prevent the onset of disease or lessen the severity of disease upon infection with the pathogen. In the setting of cancer, a vaccine is used to actively immunize the host against a tumor antigen or antigens with the intention to treat existing disease, for example, to eliminate residual undetected micrometastases after removal of a primary tumor or to induce regression of advanced, clinically evident metastases.

The immunology and molecular biology that serve as the foundation for advances in the development of cancer vaccines are complex and a detailed review is beyond the scope of this summary. In essence, both antibody- and cell-mediated immune responses against tumor antigens may be important for mediating tumor regression, although the relative contribution of each is unknown. A majority of cancer vaccines introduced into the clinic in the past several years have been designed to induce cell-mediated antitumor immune responses for the following reasons: (1) It is now understood that T lymphocyte receptors recognize peptides that sit in the groove of major histocompatibility complex (MHC) molecules on the surface of the target or antigen-presenting cell (APC). The peptides are derived from processing of intracellular proteins and are loaded onto MHC molecules in intracellular compartments. Thus, intracellular proteins can serve as tumor antigens for cell-mediated immune responses. (2) Many mutant or aberrant intracellular proteins have been identified and characterized in malignant cells, for example, mutant oncogenes or tumor suppressor proteins, or novel proteins that result from chromosomal translocations. These proteins containing unique sequences are less likely to have induced immunologic tolerance, and therefore are more likely to be recognized by a host antitumor immune response. (3) Many animal tumor models, particularly those involving therapy with vaccines or cytokines, have demonstrated that antitumor activity is mediated by a cell-mediated response, particularly T lymphocytes.

There are essentially three basic approaches for inducing antitumor immune responses in cancer patients, and almost a limitless number of variations on these approaches. The first and most traditional of the approaches involves *ex vivo* manipulation of autologous or allogeneic tumor cells as the source of cancer antigens for vaccines. The vaccine that is administered to the patient is composed of the whole tumor cells or lysates of the tumor cells that are combined with an immunologic adjuvant. In the past several years the whole tumor cell vaccines have been modified to express cytokine or other genes that increase the immunogenicity of the cell preparation.

The second approach, in essence a variation of autologous tumor cell vaccines, is to induce tumor antigen presentation *in vivo* while avoiding any *ex vivo* manipulation of tumor. For the latter, agents are injected directly into the tumor to convert the tumor cell into a proficient antigen-presenting cell or to attract antigen-presenting cells to the tumor site. Agents can also be administered systemically to stimulate *in vivo* antigen presentation, for example, by increasing the number and function of professional antigen-presenting cells.

Both of the first two approaches to immunization against cancer have in common the presentation of mostly undefined tumor antigens derived from whole tumor cells. The third basic approach to inducing antitumor immune responses is distinct in that it involves immunization with defined tumor-specific or tumor-associated antigens. The tumor antigen in the vaccine can be administered in the form of the immunogenic epitope of the antigen, the entire antigen, or as a gene for the antigen contained in a viral or nonviral gene delivery vehicle. The three types of cancer vaccine approaches that are described above are discussed in greater detail in the sections that follow.

Immunization with Autologous or Allogeneic Tumor Cell Preparations

Immunizing patients with preparations of autologous or allogeneic tumor cells has the potential of inducing immune responses against multiple antigens. This is desirable because a broad-based immune response against multiple antigens would be predicted to address the problem of heterogeneous tumor cell populations that give rise to “resistant” antigen-loss variants.

Autologous tumor cell vaccines have been preferred over allogeneic preparations since the former may contain patient-specific tumor antigens formed from random or nonrandom gene mutations. Administering these whole-cell vaccines obviates the need to define all potential tumor antigens, a task that would not be possible or practical to accomplish individually for each patient with current technology.

Unfortunately, there are substantial regulatory, pharmaceutical, practical, and cost impediments when attempting to harvest tumor and create a unique vaccine *ex vivo* for each individual patient. In addition to the practical problems, the amount of antigen presented and form of antigen in cellular vaccines are difficult to control, possibly resulting in suboptimal immunization. Whole tumor cells used for immunization are also capable of producing immunosuppressive substances (for example, certain cytokines) that may impede development of optimal antitumor immune responses. Finally, because the tumor antigens in the cellular vaccines are not fully defined, monitoring of immunologic response to the vaccine is more difficult and less precise, complicating the evaluation of the vaccine in early clinical trials.

Several autologous and allogeneic tumor cell vaccines have been studied in clinical trials over the past 20 years, usually administered with nonspecific immune adjuvants such as BCG, DETOX, or alum. Objective regression of limited metastatic disease was observed in a small percentage of melanoma and renal cell carcinoma patients receiving these types of cancer vaccines. The vaccines were also reported to improve survival in comparison to historical controls when administered to patients with metastatic melanoma, or melanoma patients at high risk for recurrence following resection of the primary tumor. Improved survival was similarly reported for patients with fully resected colorectal carcinoma in a small randomized trial comparing an autologous colon cancer vaccine with observation. Unfortunately, in the limited circumstances where these vaccines have been tested in larger randomized controlled trials, the therapeutic effects could not be confirmed. Additional phase III trials are in progress or planned.

A large number of preclinical studies have shown that insertion of cytokine or other genes (including CD80 or B7.1, a T cell costimulatory signal, the cytokines IL-2, IL-4, IL-12, TNF, GM-CSF, and interferon-gamma, and foreign HLA alleles such as HLA-B7 in HLA-B7-negative patients) into tumor cells (or cells in the vicinity of the tumor) can enhance the immunogenicity of these cells when administered as a tumor vaccine. In general, the transfer of genes to tumor cells is intended to enable presentation of tumor antigen directly to T lymphocytes or to create a local milieu conducive to antigen presentation (for example, by activating the body's own professional antigen-presenting cells and promoting their migration into the tumor). The vaccines have been shown to be effective for inducing resistance to a challenge by parental nontransfected tumor cells or causing regression of micrometastatic tumor. Although a large number of clinical trials have been initiated, only a few have been completed and published. Clear evidence that the transfected gene in these vaccines is enhancing the induction of immune responses to tumor antigens, or that the vaccine confers overall clinical benefit, is not yet available.

Vaccine Approaches That Involve Induction of Immune Responses to Tumor Antigens *In Vivo*

As noted, a major disadvantage to preparation of autologous tumor cell vaccines is the requirement for tumor biopsies and *ex vivo* manipulation of tumor cells. An alternative is to administer agents that will lead to tumor antigen presentation *in vivo* without *ex vivo* manipulation of cells. With this approach, a defined uniform pharmaceutical product is administered to the patient, thus bypassing the many regulatory, cost, and practical limitations of *ex vivo* prepared tumor cell vaccines. In some respects, this type of vaccine approach is a modernization of the now seldom-used technique of direct intratumoral administration of BCG for melanoma.

Various substances can be injected into tumors that will enhance antigen presentation either directly by the tumor, or will promote antigen uptake and presentation by the host's professional APC. For example, viral or other types of gene transfer vectors can be used to deliver cytokine or T cell costimulatory genes into tumors that will result in antigen presentation and will perhaps lead to a systemic antitumor immune response. First-generation agents include vaccinia viruses containing the costimulatory molecule B7.1 or the cytokines IL-12 or GM-CSF. In some circumstances, introduction of a foreign MHC allele into tumor (an HLA-B7 gene in HLA-B7-negative patients) may be sufficient to trigger an inflammatory response that will in turn create a local milieu conducive to the development of tumor antigen-specific immune responses. The latter approach has been reported to cause regression of injected melanoma lesions, and in at least one patient a noninjected distant visceral lesion also regressed. Finally, instead of intratumoral administration of a particular agent, it may be possible to induce antitumor immune responses *in vivo* by systemic administration of certain cytokines. For example, the cytokine flt-3 ligand has been shown to markedly increase the number of dendritic cells (APC) in multiple body tissues, and has produced tumor regression in a murine tumor model through induction of antitumor immune responses.

Defined Tumor Antigen Vaccines

The clinical application of defined tumor antigen vaccines is possible in settings where tumor antigens are shared (present on tumors from different patients), or with somewhat more difficulty, when the tumor-specific antigen from an individual patient can be rapidly identified and produced for treatment of that patient. There are both scientific and practical advantages to defined tumor antigen vaccines. Antigens in these vaccines can be easily manipulated in amount or type of presentation to maximize immunogenicity. Since the antigen is known, laboratory monitoring of the vaccine immunologic effects is simplified. Finally, a defined antigen vaccine is inherently a uniform and well-characterized product and is preferred for various pharmaceutical and regulatory reasons. The major disadvantage to these vaccines is the concern that the antigens recognized to date (i.e., mostly shared antigens) may be weaker antigens in comparison to those that result from patient-specific mutations. Also, immunizing with one or a few antigens increases the possibility that antigen-loss tumor variants will escape the immunologically mediated therapeutic effect.

A large number of tumor-specific or tumor-associated antigens have been identified. Although no strict classification system exists, it is convenient to consider antigens that are primarily intended to induce antitumor antibody responses separately from those intended to generate T lymphocyte responses. Antigens that induce primarily serologic immune responses are most often cell surface carbohydrates. For optimal immunogenicity in patients, some of the carbohydrate epitopes have been conjugated to a large antigenic protein carrier such as KLH and admixed with a nonspecific immune adjuvant. Examples include the Stn carbohydrate epitope from mucins found on many adenocarcinomas, and the GM2 ganglioside expressed by melanoma.

A small randomized trial in stage III melanoma patients (at high risk for recurrence following resection of the primary tumor) of a GM2 vaccine admixed with BCG adjuvant, compared to observation, produced a disease-free and overall survival advantage in patients receiving the vaccine, although the results were of borderline statistical significance. The GM2 was subsequently modified by conjugation to KLH and the conjugate was admixed with the QS-21 adjuvant in a phase I trial. The modified vaccine produced higher and more prolonged immunoglobulin (IgG) titers against GM2, leading to a phase III randomized trial conducted by the Eastern Oncology Cooperative Group, also for stage III melanoma patients. Induction of antibody responses to cell-surface tumor antigens *in vivo* is also being attempted with anti-idiotype antibodies (antibodies to the variable region of antibodies against the original antigen). The anti-idiotype antibodies contain the physical image of the tumor antigen and can induce antibody responses that recognize the target antigen as expressed on tumor cells.

The majority of defined tumor antigens incorporated into cancer vaccines have in common the property that they can potentially be recognized by T lymphocytes. In some cases, particularly for melanoma, the tumor antigens were identified as targets of well-characterized T lymphocyte responses to tumor cells that were detected in biopsies or peripheral blood of patients with advanced disease. Several of these antigens were found to be normal differentiation proteins restricted to the melanocyte lineage, for example, gp100, MART-1, and tyrosinase. Others were demonstrated to be present in melanoma and some other malignancies, with normal tissue expression limited to the testis. Examples of the latter type of antigens include MAGE-1 and MAGE-3, and antigens from the BAGE and GAGE families.

In contrast to the selection of antigens for vaccine studies that are known targets of a human T lymphocyte response, some antigens have been incorporated into vaccine clinical trials primarily on the basis of their specific expression in tumor or overexpression in tumor compared to normal tissue. Presumably, the restricted expression of the antigen, or the fact that the antigen is "non-self" because it is a viral protein or is derived from a mutation or chromosomal translocation, increases the probability that immune tolerance can be broken and, consequently, that an effective immune response can be raised to recognize the antigen. Potential tumor-associated antigens that are normal proteins with limited normal tissue expression include, for example, CEA and PSA. The mutant portions of the ras and p53 proteins present in many tumors and the novel protein sequences that form as a result of chromosomal translocation are examples of potential tumor-specific antigens. Mutant ras is a particularly attractive tumor-specific antigen since the mutations occur only in specific areas of a protein (for example, in the case of K-ras, mutations are found primarily in positions 12, 13, and 61 of the protein), so that it is possible to preproduce a limited number of proteins or peptides that contain the different mutations for use in the majority of patients. Other presumably tumor-specific antigens include viral proteins that are causally related to tumor formation (for example, papillomavirus E6 and E7 in cervical carcinoma) and the unique antibody idiotypes found in lymphoma or myeloma malignant B cell clones.

Table 1 gives a partial list of defined tumor antigens and the various approaches for immunization with each antigen in clinical trials. The data are not yet mature enough to determine the optimal method of immunization with any particular antigen. For this reason, most vaccines have not yet been subjected to phase II single-arm efficacy trials in uniform patient populations, and phase III randomized trials in the surgical adjuvant setting (i.e., after resection of the primary tumor) will probably not be considered until sufficient immunogenicity and perhaps some evidence of clinical activity are demonstrated in the early developmental trials. The early results, particularly for the melanoma peptides MART-1 and gp100-209 administered in incomplete Freund's adjuvant, show that the peptide vaccines can variably induce and expand cytotoxic T lymphocyte responses *in vivo*. Some evidence of tumor response has been observed in patients with advanced disease receiving these peptide vaccines; however, there has not been a clear correlation between laboratory measurements of immune response and clinical tumor regression. None of the vaccines appear to induce toxic effects other than mild local skin reactions.

Problems and Concerns in Cancer Vaccine Development

Perhaps the major problem in most cancer vaccine development is the lack of a clear endpoint in the early clinical trials, with the exception of vaccines that induce antibody responses. Assays to detect and quantify T lymphocyte responses *in vitro* are not fully developed, and those assays that exist are only semiquantitative and are labor-intensive. Furthermore, the biologic effects that are responsible for immune-mediated tumor regression are poorly understood, variable, and complex and are probably not fully measured with the current assays.

Given the state of immune monitoring, emphasis in the assessment of vaccines in early clinical trials is given to clinical endpoints, for example, tumor regression in advanced disease. The dilemma for development is the evidence from animal models that advanced disease may itself cause immunosuppression (preventing the induction of an immune response) and for many reasons is poorly responsive to immunotherapy. Attempts to assess efficacy in patients with earlier stages of disease using progression-free or overall survival present the problem that these endpoints are subject to substantial selection bias in the patient population and can only be accurately determined in randomized controlled trials.

Finally, it must be recognized that tumors have multiple mechanisms to defeat or escape immune detection. For example, tumors may lose antigen expression or presentation, or they may express molecules or cytokines that suppress or kill infiltrating lymphocytes. Tumors may also present physiologic barriers to penetration by antibodies or lymphocytes. At the very least, these

Table 1.

Defined Antigen Cancer Vaccines

Antigen	Form of Antigen	HLA-Restriction	Method of Delivery	Disease
MART-1	peptide; fowlpox-protein; vaccinia-protein; adenovirus-protein	HLA-A2	peptide with Montanide ISA 51 adjuvant; on dendritic cells; viral constructs IM or SQ	melanoma
gp-100	various peptides and modified peptides; fowlpox-protein; vaccinia-protein; adenovirus-protein; DNA gene gun	HLA-A2 HLA-A3	peptide with Montanide ISA 51 adjuvant; on dendritic cells; viral constructs IM or SQ	melanoma
tyrosinase	peptides	HLA-A2 HLA-A24	peptide with Montanide ISA 51 adjuvant	melanoma
TRP-1 (gp75)	peptide	HLA-A31	peptide with Montanide ISA 51 adjuvant	melanoma
MAGE-3	peptide	HLA-A1	combined with pan HLA-DR helper peptide plus Montanide ISA 51	melanoma, breast, others
CEA	vaccinia-protein 180 kD; vaccinia-protein 70 kD; Alvac-protein; peptide	peptide restriction HLA-A2	peptide with various adjuvants; viral vectors ID or SQ	colon and other GI malignancies, breast, lung
PSA	vaccinia-protein peptide	peptide restriction HLA-A2	ID or SQ; peptide with various adjuvants	prostate
her2-neu	peptide	HLA-A2	peptide with Montanide ISA 51 adjuvant	breast, ovary, prostate, others
mutant K-ras	3 peptides (pos 12 mutations); 4 proteins (3 pos 12 mutation, 1 pos 13 mutation)	unknown	peptides with DETOX; proteins with QS-21	pancreas, colon, others
mutant p53	peptides individually determined	unknown	loaded on PBMC and administered IV	many
pax3-fkhr translocation	peptide	unknown	loaded on dendritic cells	alveolar rhabdo-myosarcoma
ews-fli-1 translocation	peptide	unknown	loaded on dendritic cells	Ewing's sarcoma
papillomavirus type 16 E6	peptide	HLA-A2	loaded on PBMC and administered IV	cervical
papillomavirus type 16 E7	peptide 12-20; lipopeptide 86-93 (palmitic acid tails on pan-DR helper peptide linked to CTL epitope)	HLA-A2	peptide loaded on PBMC and administered IV; with Montanide ISA 51; lipopeptide SQ	cervical
lymphoma / myeloma idiotypes	idiotypes individually determined	unknown	conjugated to KLH and given with GM-CSF; with QS-21	follicular lymphoma, myeloma

factors suggest that a broad-based immune attack against multiple antigens, and perhaps utilizing both cell-mediated and serologic responses, should be the goal of vaccine development for a particular disease. It also suggests that the efficacy of vaccine approaches in advanced disease will be limited and should not necessarily dissuade consideration for trials in minimal residual disease settings.

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IMMUNOLOGIC BASIS OF VACCINE RESEARCH AND DEVELOPMENT

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Strengthening the immunological foundation of vaccine research is now an especially important goal as vaccine programs must tackle diseases such as HIV / AIDS, hepatitis C, tuberculosis, and malaria where natural immune responses usually fail and there is little empirical guidance for vaccine development. Although basic immunology research has theoretical and practical importance for the design and development of effective vaccines, to accomplish this end increased interactions between basic immunologists and vaccine researchers need to be encouraged.

The Innate Immune System and the Basis of Adjuvant Activity

Among the indispensable tools of experimental immunologists and practical vaccine researchers are adjuvants, mixtures of substances unrelated to the immunizing antigen, usually including bacterial components, needed to provoke effective immune responses to many noninfectious antigens. Current evidence indicates that adjuvants exert their activity mainly by activating cells of the innate immune system, the first line of defense against invading microbes, which consists of cells positioned in the skin and mucosal tissues. These cells, which include macrophages, Langerhans cells, tissue dendritic cells, and epithelial cells, express receptors dedicated to the recognition of molecules found in microorganisms. These are typically lipids and polysaccharides that are absent in mammals but widely distributed in many bacteria, fungi, and protozoa.

Examples of receptors involved in these recognition processes include the lipopolysaccharide receptor, glycan receptors, and macrophage scavenger receptors. The full range of such receptors and their complete function are not yet known, but current evidence indicates that they act as sentinels to detect the presence of microbial invaders. They appear to serve two major roles. First, they trigger cells to take immediate defensive action against microbial invaders, such as phagocytosis of the pathogen and secretion of antimicrobial compounds. Second, and relevant to adjuvant activity, they set in motion a series of events that activate the adaptive immune system.

Strong evidence for this latter role was provided by a recent finding of a human gene closely related to defensive molecules found in plants and insects that signals the immune system. This gene is the human homologue of *Toll*, a molecule known to be involved in innate immunity to fungal infections in drosophila. The human homologue hToll is expressed mainly by macrophages. The signaling portion of the molecule was shown to lead to upregulation of the B7.1 protein, a molecule especially important in costimulating helper T cells that are responding to antigen.

This finding provides an example of an important link between innate and adaptive immunity that could be exploited for designing new adjuvants. For example, once the ligand that binds the ectodomain of hToll is identified, it should be possible to synthesize compounds that will bind and activate hToll specifically, potentially a novel adjuvant for vaccines. It may be anticipated that many receptors with different specificities for pathogen-associated molecules and different signaling capabilities await discovery. They may offer new targets for the rational design of adjuvants.

Memory T Cell Activation and Trafficking *In Vivo*

Immunological memory, the rapid and potent immune response to a previously encountered antigen, is an important component of vaccine effectiveness. Once B and T lymphocytes have been stimulated in an immune response, some cells, by mechanisms not well understood, are maintained for long periods as memory cells. New observations were reported from studies using an *in vivo* system whereby T cells with known T cell receptors from transgenic mice were transferred into normal recipients where they could be followed with appropriate markers as they participate in immune responses. These studies found that for memory T cells to be induced, the antigen must be administered so as to elicit production of the cytokines tumor necrosis factor alpha and interleukin-1. These cytokines are induced when antigen is injected emulsified in complete Freund's adjuvant, which contains killed mycobacterial cells, but not if soluble antigen is administered in saline.

Besides being available for responding to future infections, memory T cells also respond more rapidly to antigen and secrete a different cytokine profile than naïve T cells. Memory cells typically secrete much greater amounts of interleukin-2 and interferon-gamma than primary T cells do. An important new observation is that memory T cells have the enhanced capacity to migrate to regions of lymph nodes rich in immunoglobulin-producing B cells. Potentially, this increases the probability of T cell interaction with B cells and the delivery of T cell help for specific antibody production. Thus, memory T cells differ significantly from those encountering antigen for the first time, in ways that explain longstanding observations about the speed and vigor of recall immune responses. Importantly, many vaccines fail to provide life-long immunity and require periodic boosting to maintain immunological memory. Better understanding of the induction, maintenance, and triggering of memory T cells may permit development of vaccines providing longer protection.

Targeting Antigens for Inducing Cytotoxic T Cells

Vigorous CD8+ cytotoxic T cell (CTL) responses are an important determinant of protective immune responses to many viruses and intracellular bacteria. CTL recognize antigen as small fragments of foreign proteins, proteolytically processed to peptides of eight or nine amino acids, and presented in the alpha-helical groove of the class I major histocompatibility complex (MHC) molecule. Research from several laboratories has shown that the key to class I MHC antigen presentation is to introduce the antigen into the cytoplasm of antigen-presenting cells (APC). There, the antigen can be acted on by a pathway that involves degradation into peptides by action of proteosomes (a large complex of proteolytic enzymes) and uptake into the endoplasmic reticulum by a specific transport molecule, where peptides can then associate with nascent class I MHC molecules. Peptides that associate with sufficient strength will stabilize the class I MHC molecule, which is then transported to the surface of the APC where the complex is available for interaction with T cell receptors. In addition, the so-called "professional" APC have the ability to express molecules such as B7.1 and B7.2 that deliver costimulatory signals to T cells while they interact with antigen, a requirement for inducing T cell activation. Although many infectious viruses and DNA plasmids have the ability to gain access to the cytoplasm of professional APC, potentially much safer vaccines could be made if purified proteins or peptides could be induced to enter the cytoplasm.

A method was found that exploited the phagocytic function of key APC such as macrophages and dendritic cells. Proteins coupled to micron-sized beads were engulfed by phagocytic cells, resulting in a very efficient presentation of the associated antigens in context of class I MHC. Further, class II MHC-restricted helper T cell responses were also induced by this method, which may be important for obtaining optimal CTL induction. Importantly, the procedure was found to work well *in vivo*, resulting in strong CTL responses when antigen-coupled beads were injected without adjuvant. Data from a tumor model indicate the method induces CTL capable of biological activity *in vivo*. This approach may form the basis of a new type of vaccine for CTL responses, which could be further enhanced with modifications such as incorporating proteolytic cleavage sites into the antigen for more efficient proteolytic processing and transport, or inclusion of peptides for helper T cell responses.

T Cell Epitope Immunodominance and Its Role in Shaping Cytotoxic T Cell Responses

T cell responses to protein antigens are focused on relatively few "immunodominant" determinants. This limitation in T cell responses may facilitate the escape of pathogens from T cell surveillance via the mutation of only a few regions of key proteins; therefore, it may be advantageous to design vaccines to circumvent immunodominance, potentially eliciting a more broad CTL attack that is less likely to select escape variants. Many factors can contribute to a T cell epitope emerging as immunodominant. While it might be anticipated that an extraordinarily high level of cell-surface expression of a specific peptide-MHC complex might be an essential requirement, analysis of the immunodominant epitope used in the mouse MHC class I H-2K^d CTL response to the influenza virus nucleoprotein reveals that this need not be true. The peptide that stimulated by far the best T cell response bound H-2K^d at least tenfold less well than three other T cell antigenic peptides derived from the same antigen. Further, the immunodominant determinant was presented by virus-infected APC at relatively low numbers of complexes per cell, ruling out the possibility that abundance might have compensated for low MHC binding avidity in this case.

Recent results suggest that immunodominance by this determinant primarily derives from the very high avidity of the antigen receptors of T cells that predominate in the response to this peptide:MHC complex. Therefore, the strength of T cell recognition appears to be able to compensate for shortcomings, both in MHC-binding avidity and antigen presentation, to result in an immunodominant determinant. In addition, some form of direct or indirect T cell control over T cell responses may also contribute to immunodominance in this case. T cell responses to the immunodominant nucleoprotein region were found to somehow interfere with responses to subdominant determinants.

Immunization of mice with the wild-type nucleoprotein elicited mainly the immunodominant response, while injection of a mutant nucleoprotein that lacked the immunodominant epitope permitted development of subdominant T cell responses. It is currently unclear whether this reflects competition between T cells, possibly for attachment and costimulation by APC presenting specific antigen, or whether a more active form of suppression is occurring. Knowledge of mechanisms of immunodominance should permit development of vaccination approaches to induce broader T cell responses to target antigens. However, research is also needed to determine whether certain aspects of immunodominance, such as regulation of T cell responses to subdominant determinants, serve an important immune control function that may be deleterious to the host if upset.

Genetic Nonresponsiveness to Vaccination

The presence of a significant fraction of nonresponders to a particular vaccine can undermine the effectiveness of that vaccination program, both on the population level by failure to achieve target levels of protection, as well as on the individual level by inappropriately reducing avoidance or protective behavior, thereby increasing the potential for contact with the disease. Approximately 5 percent of those receiving the three-injection course of recombinant hepatitis B surface antigen vaccine (HBsAg) fail to generate protective antibody and T cell responses, although they respond well to other vaccines.

Genetically, responsiveness to HBsAg is a dominant trait. Nonresponders may more frequently express the class II MHC molecules DR3 and DR7, but this is not absolute. However, assays of peripheral blood T cells clearly distinguish responders, which strongly proliferate in presence of HBsAg in culture, from nonresponders, which do not. T cells from both groups of donors respond to tetanus toxoid and mitogens, and defects in antigen-presenting cells or presence of suppressor cells can be ruled out. Defects in T cell activation may therefore underlie nonresponsiveness. Conceivably, nonresponders may lack certain T cell receptor genes needed to recognize HBsAg epitopes, or, alternatively, the defect may occur after antigen recognition, related to signal transduction in the T cell.

Recent evidence that diverse T cell receptors are used by responder individuals to recognize HBsAg may make the explanation of "holes" in the T cell receptor repertoire less likely; however, further information on T cell receptor usage related to genotype, as well as discovery of additional genetic associations with nonresponsiveness, is needed. Research in this area should be especially relevant for peptide or protein vaccines that comprise a limited number of T cell epitopes, which may exaggerate individual differences in responses.

More active collaboration and increased communication between basic immunologists and clinical vaccine researchers and microbiologists have the potential to improve vaccines greatly. There is an increased need for rationally based approaches to vaccine design and development, which could assist in circumventing limitations of empirical approaches and lead to greater understanding of mechanisms of vaccine action. Basic immunologists can play an important role in this regard, because of their emphasis on underlying mechanisms and facility with animal models and manipulation of immune responses.

Fundamental immunological principles can be used to guide the design and development of new compounds and methodology, such as more effective adjuvants, methods to enhance humoral versus inflammatory immune responses, and development of new techniques to monitor antigen-specific cellular immunity in humans. Effective strategies also need to be formulated by clinical and basic researchers to understand the causes of nonresponsiveness to vaccines, the function of the immune system in chronic infection, and development of therapeutic vaccines to treat ongoing disease. Many innovative approaches to these problems could be formulated by active interactions of clinical vaccine researchers with basic immunologists.

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VACCINES FOR IMMUNOLOGIC DISEASES

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Background

Vaccines are the most effective medical intervention for preventing infectious diseases. Originally, the definition of vaccine was restricted to vaccinia preparations used for immunization. Over time, the term has evolved to include all preparations used to generate protective immunity to microbial pathogens or their toxins. More recently, the definition of vaccine has been further expanded to include antigenic materials used to tolerize—or turn off—antigen-specific immune responses to prevent or treat immune-mediated diseases. The following discussion highlights the status of recent efforts to develop tolerogenic vaccines for immune-mediated diseases and identifies promising areas of further research.

Autoimmune diseases affect nearly 5 percent of adults in North America and Europe, and allergic diseases affect more than 15 percent of children and adults. These chronic relapsing disorders affect multiple organ systems and are characterized by substantial morbidity and mortality and by their high social and economic costs. Vaccines that could prevent or treat these conditions would be an important addition to existing therapies.

The properties of immune tolerance and the underlying mechanisms have not been clearly defined, but the following are important considerations: (1) tolerance refers to a selective inability to respond to antigens—this can be a learned phenomenon or due to immunological ignorance; (2) both foreign and self-antigens can be targets of tolerogenic processes; (3) although tolerance can be mediated by suppressor cells, tolerance is not the same as immune suppression, either mechanistically or clinically; (4) tolerance can be maintained by active or passive processes and can result from cell inactivation, altered function, or death; and (5) tolerance can be induced centrally (in the thymus) or peripherally.

The activation of T cells is central to virtually all autoimmune events. Tissue injury in autoimmune diseases is mediated by cytotoxic T cells, neutrophils, and macrophages, or through the actions of autoantibodies and complement. T cells play a central role in all of these events, by providing "help" for antibody production and by producing secretory products that activate and recruit phagocytic cells to sites of inflammation.

A variety of approaches are being pursued to induce T cell tolerance. These include blocking the activation of T cells by antigen presenting cells, focusing on the interactions of the T cell receptor (TCR) with peptides presented by the major histocompatibility complex (MHC). Other strategies target costimulatory pathways in T cells, or the interactions of cell surface adhesion molecules and their counter-ligands. Some of these experimental therapies are now being developed as candidate vaccines.

Mechanisms of Tolerance

Immune responses in the gastrointestinal tract are self-limited, and repeated challenge with certain antigens results in a diminished response. Oral administration of both high- and low-dose antigen results in a phenomenon termed "oral tolerance," in which the immune response to subsequent systemic administration of antigen is blocked. At least two mechanisms appear to be important. Tolerance to high-dose antigen appears to be via inactivation or clonal deletion of Th1 and Th2 cells. In contrast, tolerance to low-dose antigen is really "bystander" immune suppression mediated by stimulation of Th2- and Th3-type cytokines, with TGF-β being the major suppressive cytokine in various model systems. Other routes of mucosal tolerance have recently been explored, including immunization via the nasal and respiratory mucosa. These routes appear to be equally or more efficient in inducing immune tolerance in animal models. By eliminating enzymatic degradation in the gastrointestinal tract, nonoral routes of immunization offer the theoretical advantage that lower doses of antigen will be needed.

In human autoimmune diseases there are often reactivities to multiple autoantigens in target organs. This intra- and interantigenic spread—"epitope spreading"—is a characteristic of chronic inflammation in autoimmune disorders. Bystander suppression refers to the generation of regulatory cells (first demonstrated after oral administration of myelin basic protein) that nonspecifically suppress inflammation in the target organs where the fed antigen is present. Thus, it may be possible to design vaccines that prevent organ injury even in those diseases where all of the relevant autoantigens are not known. As such, bystander suppression solves a major conceptual problem in developing vaccine-based therapies for many autoimmune diseases.

Different mechanisms appear to be important in other model systems. For example, T cell inactivation results when antigen is presented to the T cell, but costimulatory signals, also called second signals, are blocked. T cells made tolerant in this way will not respond when re-presented with the same antigen, even in the absence of costimulatory blockade. Generation of costimulatory signals depends on cognate interactions between B7 molecules and CD40 on antigen-presenting cells and their counter-receptors, CD28 and CD40 ligand, on the surface of T cells. Numerous approaches to block these interactions are now being explored.

In certain model systems, differences in Th1 and Th2 responsiveness correlate with the development of autoimmune (Th1-predominant) and allergic (Th-2 predominant) reactivity. In addition, immune deviation from a Th2 to a Th1 predominance also creates a barrier to long-term allograft acceptance. In humans there

appears to be a greater plasticity of T cell responses, as opposed to the more polarized Th1/Th2 demarcation seen in the mouse. Nonetheless, in addition to vaccines that induce global tolerance, vaccines that could direct immune responses toward a Th2 predominance might be of value in autoimmune diseases.

Clinical Opportunities and Clinical Trials

Disease prevention should be the goal when at-risk individuals can be identified before significant injury has occurred (e.g., to prevent transplant rejection or for immune-mediated diabetes mellitus before destruction of insulin-producing islets). In other settings (e.g., rheumatoid arthritis), clinical improvement might still be possible even though at-risk individuals cannot be identified before the onset of disease.

The approach that has been most widely used is to tolerize pathogenic T cells by oral or systemic administration of the target antigen. The identification of disease-related antigens is a prerequisite for such an approach. The importance of certain autoantigens is clear because the presence of antigen-specific antibodies or T cells correlates with disease activity, and the disease can be mimicked in animal models via adoptive transfer of autoantibody or autoreactive T cells.

Examples include insulin and the enzyme glutamic acid decarboxylase (GAD) in immune-mediated diabetes mellitus, myelin basic protein (MBP) in multiple sclerosis (MS), and the keratinocyte cell adhesion molecule desmoglein 3 in pemphigus vulgaris. The evidence pointing to MBP as a target antigen in MS is substantial: (1) MBP causes an MS-like illness when injected into animals; (2) MBP-specific cells are found at increased frequency among the circulating activated T cells of MS patients; (3) MBP-specific T cells have been localized to lesional sites in the brain; and (4) an MS-like disease can be induced in immunodeficient mice (which do not reject human cells) by MBP-reactive T cells from the cerebrospinal fluid of patients with MS.

Self-antigens have been administered as tolerogenic vaccines in animal models of human autoimmune diseases including rheumatoid arthritis, insulin-dependent diabetes mellitus, multiple sclerosis, and uveitis. In most of these studies, nonresponsiveness was induced by administration of the self-antigen via the oral route. These approaches have been successful for treatment and prevention in animal models. However, the observed responses have generally been less than complete, characterized largely by delays in onset of disease or reductions in disease severity.

Based on promising results in animal models, pilot open trials or blinded randomized studies have been initiated in insulin-dependent diabetes mellitus (oral administration of insulin), multiple sclerosis (oral administration of bovine myelin), rheumatoid arthritis (oral administration of chicken type II collagen), and uveitis (oral administration of bovine retinal S antigen). Other trials are in progress or are planned. Taken as a group, these trials have shown safety with relatively few side effects. Clinical improvements have been seen in some studies, but enthusiasm has also been tempered by recent failures.

In contrast, the goal of immunotherapy for atopic diseases is to drive immune responses in the opposite direction—toward a Th1 predominance. Standard allergen immunotherapy may do this but probably with limited efficacy. One recently developed approach involves vaccination with DNA. Plasmid DNA contains short immunostimulatory sequences that specifically promote Th1 responses to the recombinant proteins encoded by these vaccines. DNA vaccines encoding a variety of natural and "model" allergens have now been tested in animal models. In a murine model of allergic asthma, DNA vaccination leads to allergen-specific reductions in IgE, induction of allergen-specific IgG "blocking" antibodies, and a decrease in allergen-induced bronchial hyperreactivity. It is likely that clinical trials with DNA vaccines will be launched in the near future.

Another very promising approach involves immunization with short linear peptides representing the major T cell epitopes of common allergens. This approach has recently demonstrated efficacy and eliminates the major complication of standard immunotherapy, i.e., the risk of life-threatening anaphylaxis triggered by preexisting IgE (on mast cells and basophils) that is directed to epitopes of "whole" allergen.

Standard allergen immunotherapy not only causes modest reductions in interleukin-4 production by allergen-specific T cells, but also leads to production of IgG "blocking" antibodies. The role of these IgG antibodies in altering allergic responses has long remained unclear. The actions of IgG blocking antibodies may be explained by the discovery of a negative signaling pathway in mast cells and basophils. This pathway is activated by immune complexes that cross-link mast cell and basophil receptors for IgG. Turning on this negative pathway blocks the activation of these cells that would otherwise occur with cross-linking of the receptors for IgE. Novel vaccines could be developed to target more effectively this important pathway.

Future Directions

Antigen-specific immune tolerance can be induced in animal models and in human autoimmune diseases. However, many important questions remain to be answered. Key areas for future investigation include (1) further identification of disease-specific autoantigens; (2) studies to optimize vaccine delivery and antigen processing for tolerance induction; (3) characterization of costimulatory pathways and identification of new approaches for their inhibition; (4) studies of the role of the cytokine milieu in tolerance induction; and (5) development of gene transfer-based approaches for tolerance induction. With advances in these and other areas it is likely that today's promising leads can be developed into effective vaccines.

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APPENDIX A

Status of Vaccine Research and Development, 1998

Target Agent	Vaccine	Basic R&D	Preclinical	Phase I	Phase II	Phase III
Ancyclostoma duodenale	Recombinant protein	+	+			
Bacillus anthracis	Recombinant subunit	+	+			
Bordetella pertussis	*B. pertussis* surface protein expressed by vector (e.g., *Salmonella* and *Vibrio cholerae*)	+	+			
	Purified PT vaccine—acellular	+	+	+	+	+
	Recombinant PT vaccine—acellular	+	+	+	+	
	Purified PT and FHA—acellular	+	+	+	+	+
	Purified PT, FHA, pertactin, and agglutinogens 2 & 3—acellular	+	+	+	+	+
	Purified PT, FHA, pertactin—acellular	+	+	+	+	+
	Recombinant PT, FHA, pertactin—acellular	+	+	+	+	+
	PT peptides-CRM conjugates	+	+			
	Purified adenylate cyclase	+	+			
	DTP-Hib conjugate	+	+	+	+	+
	DTP-Hib conjugate-HBV	+	+	+	+	+
	DTP-IPV	+	+	+	+	
	DTP-Hib-conjugate-IPV-HBV	+	+	+	+	
	DTaP-Hib conjugate-HBV	+	+	+	+	
	DTaP-IPV—monovalent aP	+	+	+	+	
	DTaP-Hib conjugate-IPV-HBV—bivalent and trivalent aP	+	+	+	+	
	DTaP-Hib	+	+	+	+	+
Blastomyces dermatitidis	Purified yeast cell proteins (e.g., WI-1)	+	+			
	Recombinant proteins (e.g., WI-1)	+				
Borrelia burgdorferi	Recombinant Osp A	+	+	+	+	+
	Osp A-based DNA vaccine	+	+			
	BCG-expressed Osp A	+	+			
	Purified Osp B, Osp C	+	+			
Brugia malayi	Purified parasite antigens (paramyosin, etc.)	+	+			
Campylobacter jejuni	Inactivated whole cell with mutant *E. coli* labile toxin (mLT) adjuvant, oral vaccine	+	+	+		
	Whole cell (intact)	+	+	+	+	
Chlamydia pneumoniae	Purified, major outer membrane protein, heat shock protein	+				
Chlamydia trachomatis	Major outer membrane protein (MOMP) viral vectors	+	+			
	Purified major outer membrane protein	+	+			
Clostridium botulinum	Toxoid	+	+	+	+	

+ indicates that studies have been completed or are in progress.

The vaccines and vaccine approaches listed on this table represent those vaccines known to NIAID scientific program staff at the time of publication of the 1998 *Jordan Report*. Readers of this report who are aware of additional information that should appear in future editions are encouraged to notify the editor.

Target Agent	Vaccine	Basic R&D	Preclinical	Phase I	Phase II	Phase III
Clostridium difficile	Formalin-inactivated toxins A and B	+	+			
Clostridium tetani	Recombinant toxin	+	+			
	Salmonella vector	+	+	+		
	Microencapsulation	+	+			
Candida albicans	Cell surface oligomannosyl epitope	+	+			
Chikungunya virus	Live, attenuated	+	+	+	+	
Coccidioides immitis	Formalin-killed spherules	+	+	+	+	+
	Recombinant 7.3 kD protein	+	+			
	Spherule homogenate (27kxg)	+	+			
	33 kD protein	+	+			
	DNA vaccine (7.3 kD protein in pc DNA3)	+	+			
Corynebacterium diphtheriae	Recombinant toxin	+	+			
	Salmonella vector	+	+	+		
Coxiella burnetti	Formalin inactivated	+	+	+	+	
Cryptococcus neoformans	Partially purified capsular polysaccharide	+	+			
	Glycoconjugate of capsular polysaccharide with tetanus toxoid	+	+	+		
Cytomegalovirus (CMV)	Live, attenuated strains (conventional)	+	+	+	+	
	Live, attenuated strains (engineered)	+	+			
	Glycoprotein subunit vaccine	+	+	+	+	
	Multiprotein subunit vaccine	+				
	Nucleic acid (DNA) vaccines	+	+			
	Canarypox vectored	+	+	+		
Dengue virus	Purified rDNA-expressed viral proteins	+	+			
	Infectious clone	+	+			
	Chimeric virus	+	+			
	Inactivated whole virus particle	+	+			
	Vaccinia vector (live)	+	+			
	Vaccinia subunit	+	+			
	Baculovirus subunit	+	+			
	Synthetic peptide	+	+			
	Micelle / ISCOM	+	+			
	Yeast subunit	+	+			
	Recombinant envelope (baculovirus and drosophila expression systems)	+	+			
	Live, attenuated dengue virus (monovalent)	+	+	+	+	
	Live, attenuated dengue virus (combined quadrivalent)	+	+	+		
	DNA vaccine	+	+			
Eastern equine encephalitis virus	Inactivated whole virus particles	+	+	+	+	
Endotoxin (Gram-negative sepsis)	Detoxified lipopolysaccharide from *E. coli* 0111:B4, Rc (J5)	+	+			

Target Agent	Vaccine	Basic R&D	Preclinical	Phase I	Phase II	Phase III
Entamoeba histolytica	Yeast subunit	+	+			
	Recombinant galactose-binding protein	+	+			
	Galactose-binding proteins expressed in *Salmonella*	+	+			
Enterohemorrhagic *Escherichia coli* (EHEC) [Shiga toxin-producing *E. coli* (STEC)]	Nontoxic mutant toxins	+	+			
	Intimin	+				
	LPS conjugates	+	+			
	Intimin expression in plants	+				
	Stx-1 beta-subunit in *Vibrio cholerae* vector	+	+			
Enterotoxigenic *Escherichia coli* (ETEC)	Killed cells and beta-subunit of cholera toxin	+	+	+	+	
	Nontoxigenic ETEC derivative, live, attenuated	+	+	+	+	
	Salmonella and *Shigella* vectored CFAs	+	+			
	Subunit synthetic toxoid (ST) and B subunit of heat-labile toxin (LT)	+	+			
	LTB expressed in potatoes	+	+	+		
	CFA II microencapsulated	+	+			
Epstein-Barr virus (EBV)	Glycoprotein subunit (gp350)	+	+	+		
	Vaccinia recombinant virus expressing gp350	+	+	+		
	Peptide induction of CTL	+	+	+		
Escherichia coli (urinary tract)	Anti-FimH adhesin	+	+			
Filoviridae (Ebola)	Recombinant subunit	+	+			
	Replicons	+	+			
Francisella tularensis	Live, attenuated	+	+	+	+	
Group A streptococcus	Glycoconjugate Group A polysaccharide with tetanus toxoid	+	+			
	M protein / peptides linked to toxin subunit carriers	+	+			
	M protein, multivalent type-specific epitopes	+	+			
	M protein, type-specific epitope(s) linked to toxin subunit	+	+			
	M protein, epitope of conserved region	+	+			
	M protein epitopes expressed in commensal vectors (*S. gordonii*)	+	+			
	Cysteine protease	+	+			
	C5a peptidase	+	+			
Group B streptococcus	Glycoconjugate vaccines of type Ia, Ib, II, III, and V linked to a carrier protein	+	+	+	+	
	Capsular polysaccharide (C-beta) conjugates with types Ia, II, and III	+				
Haemophilus ducreyi	Major outer membrane protein	+	+			
	Hemolysin / cytotoxin	+	+			
	Hemoglobin receptor	+	+			
Haemophilus influenzae (nontypeable)	Recombinant protein subunit containing either P1, P2, or P6 proteins to serve as carriers in conjugate vaccine preparations	+	+			
	Recombinant protein subunit containing P4 and P6	+	+			
	Subunit Hi nontypeable 47 OMP (adjuvanted)	+	+			

Target Agent	Vaccine	Basic R&D	Preclinical	Phase I	Phase II	Phase III
Haemophilus influenzae (nontypeable) (continued)	Subunit lipoprotein D (nonacylated)	+	+			
	Subunit detoxified lipooligosaccharide conjugated to tetanus toxoid	+	+			
	Subunit detoxified lipooligosaccharide conjugated to HMW protein from *H. influenzae* (nontypeable)	+	+			
Haemophilus influenzae type b (Hib)	Glycoconjugate of Hib PRP with CRM197	+	+	+	+	+
	Glycoconjugate of Hib PRP with diphtheria toxoid	+	+	+	+	+
	Glycoconjugate of Hib PRP with tetanus toxoid	+	+	+	+	+
	Hib-IPV-HBV	+	+	+	+	
	Glycoconjugate of Hib PRP with meningococcal type B outer membrane protein	+	+	+	+	+
Hantaan virus	Vaccinia vector	+	+	+	+	
	Recombinant subunit	+				
	RNA replicons	+				
Helicobacter pylori	Recombinant H. pylori urease and cholera toxin—oral vaccine	+	+	+		
	Urease and mutant CT	+	+			
	Catalase and CT	+	+			
	Killed whole cells	+	+			
Hepatitis A virus (HAV)	Inactivated HAV particles	+	+	+	+	+
	Live, attenuated HAV	+	+	+	+	+
	Virosome-formulated inactivated HAV	+	+	+	+	+
	Viral proteins expressed by vectors (baculovirus or vaccinia virus)	+	+			
Hepatitis B virus (HBV)	HBV core protein expressed by rDNA	+	+			
	HBV proteins expressed in yeast cells by rDNA	+	+	+	+	+
	Salmonella vector	+	+	+		
	Variants	+	+			
	Generation of cytotoxic T lymphocytes	+	+	+	+	
	DNA vaccines	+	+			
	rDNA, plants	+	+			
Combined HAV / HBV vaccine	Combined inactivated components	+	+	+	+	+
Hepatitis C virus (HCV)	rDNA-expressed surface proteins and epitopes	+	+			
	Generation of cytotoxic T lymphocytes	+	+			
	Nucleocapsid	+	+			
	DNA vaccines	+	+			
Hepatitis D virus (HDV)	Synthetic peptides	+	+			
	Baculovirus	+				
Hepatitis E virus (HEV)	Expressed proteins	+	+			
Herpes simplex virus types 1 and 2	gD2 recombinant protein	+	+	+	+	+
	gD2 and gB2 recombinant protein	+	+	+	+	+
	Disabled virus (gH deleted)	+	+	+		
	DNA encoding gD2	+	+	+		

Target Agent	Vaccine	Basic R&D	Preclinical	Phase I	Phase II	Phase III
Herpes simplex virus types 1 and 2 (continued)	Heteroconjugate recombinant protein, T cell ligands with HSV-associated peptides	+	+			
	Vaccinia-vectored proteins glycoproteins	+	+			
Histoplasma capsulatum	Purified yeast cell proteins (e.g., His-62)	+	+			
	Recombinant proteins (e.g., His 62, H-antigen, hsp-70)	+	+			
Human immunodeficiency virus, HIV-1	**See Appendix C**					
Human immunodeficiency virus, HIV-2	Inactivated HIV-2	+	+			
	Live, attenuated HIV-2	+	+			
	rgp 125 or 130 (purified from virion)	+	+			
	rgp 160 (insect cells)	+	+			
	Highly attenuated, vaccinia HIV-2 gag-pol-env	+	+			
	Vaccinia HIV-2 env	+	+			
	Canarypox HIV-2 gag-pol-env	+	+			
	Salmonella HIV-2 env, gag	+	+			
Human papillomavirus (HPV)	Capsid protein	+	+			
	TA-HPV (live recombinant vaccinia) E6 and E7 (from HPV-16, and HPV-18)	+	+	+	+	
	TA-GN recombinant protein L2 and E7 (from HPV-6)	+	+	+	+	
	MEDI-501 recombinant VLP L1 from HPV-11	+	+	+		
	Quadrivalent recombinant VLP L1 (from HPV-6, HPV-11, HPV-16, and HPV-18)	+	+			
	DNA vaccine	+	+			
	LAMP-E7 (from HPV-16)	+	+			
Influenza virus	Cold-adapted live, attenuated	+	+	+	+	+
	Purified viral HA subunit	+	+	+		
	Liposome containing viral HA	+	+	+		
	Purified CTL specific peptides	+	+	+		
	Microencapsulated inactivated vaccine	+	+	+		
	Purified, inactivated viral neuraminidase	+	+	+		
	Baculovirus expressed recombinant HA subunit	+	+	+	+	
	Baculovirus expressed nucleoprotein	+	+	+		
	Transfection with nucleic acid (DNA) plasmid expressing HA subunit	+	+			
	Inactivated viral vaccines with novel adjuvants	+	+	+		
Japanese encephalitis virus	Whole, inactivated virus particles	+	+	+	+	+
	Infectious clone	+	+			
	Purified DNA expressed protein	+	+			
	Live attenuated virus	+	+	+	+	
	Vaccinia vector (live)	+	+	+		
Junin virus (Argentine hemorrhagic fever)	Live, attenuated	+	+	+	+	
Legionella pneumophila	Attenuated mutant	+	+			
	Purified bacterial surface protein	+	+			

Target Agent	Vaccine	Basic R&D	Preclinical	Phase I	Phase II	Phase III
Leishmania major	Attenuated or killed whole parasites	+	+	+	+	+
	Deletion mutagenized, attenuated parasite	+	+			
Multiple *Leishmania* spp.	Leishmanial surface antigens (gp63, 46 kD, and lipophosphoglycan)	+	+			
Measles virus	rDNA HA and fusion proteins	+	+			
	ISCOM	+	+			
	Live, attenuated	+	+	+	+	+
	High-titer live (multiple strains)	+	+	+	+	+
	Poxvirus vector (live)	+	+	+		
Moraxella catarrhalis	High molecular weight, outer membrane proteins CD, E, B1, and LBP for use in conjugate vaccines	+	+			
Mycobacterium leprae	BCG plus purified *M. leprae* antigens (35 kD)	+				
	Recombinant antigens in BCG	+	+			
	Live BCG expressing *M. leprae* antigens	+	+			
	BCG plus heat-killed *M. leprae*	+	+	+	+	+
	Heat-killed, purified *M. leprae*	+	+	+	+	+
	Live, cross-reacting atypical mycobacteria	+	+	+	+	+
	BCG	+	+	+	+	+
	Mycobacterium w.	+	+	+		
	Vaccinia virus vector expressing mycobacterial antigen	+	+			
Mycobacterium tuberculosis	BCG plus purified *M. tuberculosis* antigens	+	+			
	T cell reactive immunogens	+				
	Recombinant antigens in BCG	+	+			
	M. vaccae	+	+	+	+	
	Recombinant antigens in *M. vaccae*	+	+			
	M. tuberculosis culture filtrate proteins (CFP)	+	+			
	M. tuberculosis culture filtrate proteins and cytokines	+	+			
	Mycolic acids	+	+			
	BCG with CFP “boost”	+	+			
	Dendritic cells pulsed with for-met peptides	+	+			
	Transfected EL-4 cells	+	+			
	Recombinant *Salmonella* constructs	+				
	M. smegmatis expressing *M. tb* antigens	+				
	rBCG expressing cytokines	+	+			
	Auxotrophic mutant BCG	+	+			
	DNA vaccines	+	+			
	Auxotrophic mutant *Mycobacterium tuberculosis*	+	+			
	Live *Mycobacterium microti*	+	+			
Mycoplasma pneumoniae	Recombinant membrane-associated proteins	+	+			
	Purified outer membrane protein	+	+			
	Inactivated (heat-killed) oral vaccine	+	+	+		
Neisseria gonorrheae	Por (protein I)	+	+			
	Recombinant Por protein	+	+			
	Iron-binding protein (BPs)	+	+			
	PANS anaerobic proteins	+				
	H.8 lipoprotein	+				

Target Agent	Vaccine	Basic R&D	Preclinical	Phase I	Phase II	Phase III
Neisseria gonorrheae (continued)	LPS anti-idiotype	+	+			
	Whole cells	+	+			
Neisseria meningitidis (Group A)	Glycoconjugate with tetanus toxoid	+	+			
	Group A LOS	+				
Neisseria meningitidis (Group B)	Native outer membrane vesicle (NOMV)—intranasal route	+	+	+		
	OMP-dLPS liposome	+	+			
	Recombinant PorA outer membrane protein in liposomes	+	+			
	Outer membrane vesicles (OMV), high MW proteins, and C polysaccharide	+	+	+	+	+
	Hexvalent PorA outer membrane vesicle vaccine	+	+	+	+	
	Outer membrane vesicles (deoxycholate extracted)	+	+	+	+	+
	Recombinant transferrin binding protein (TBP1 and TBP2)	+	+			
	Recombinant low MW (NspA) outer membrane protein	+	+			
	Glycoconjugate modified polysaccharide with recombinant PorB protein	+	+			
	LOS micelle-based vaccine	+				
Neisseria meningitidis (Group C)	Glycoconjugate with tetanus toxoid	+	+	+	+	+
Neisseria meningitidis A and C	Glycoconjugate A and C with CRM197	+	+	+	+	
	Glycoconjugate A and C with DT	+	+	+		
Neisseria meningitidis A, B, and C	Combination glycoconjugate with recombinant PorB	+	+			
Neisseria meningitidis A,B, C, and W-135	Glycoconjugate with DT	+	+	+		
Norwalk virus	Norwalk virus-like particle (VLP)	+	+	+		
Onchocerca volvulus	Recombinant proteins	+	+			
Paracoccidioides brasiliensis	Purified yeast cell proteins	+	+			
	Recombinant proteins	+				
Parainfluenza virus	Cold-adapted PIV3 attenuated virus	+	+	+		
	Purified HN and F protein subunit vaccine	+	+			
	Bovine attenuated PIV3 vaccine	+	+	+		
Plasmodium falciparum	Circumsporozoite antigen-based peptide or recombinant protein	+	+	+	+	
	Circumsporozoite antigen expressed in various vectors	+	+	+		
	Circumsporozoite antigen-based DNA vaccine	+	+	+		
	Noncircumsporozoite, pre-erythrocytic antigen-based constructs	+	+			
	Merozoite surface protein-1 (MSP-1) based recombinant protein	+	+	+		
	Non-MSP-1 asexual blood stage antigens	+	+			
	25 kD gametocyte antigen recombinant protein (TBV25H)	+	+	+		
	Other sexual stage antigens	+	+			
	Peptide-based combination vaccines incorporating different stage-specific antigens	+	+	+	+	+

Target Agent	Vaccine	Basic R&D	Preclinical	Phase I	Phase II	Phase III
Plasmodium falciparum (continued)	Multivalent viral vector-based combination vaccines incorporating different stage-specific antigens (e.g., NYVAC Pf7)	+	+	+	+	
	Subunit (RTS,s)	+	+	+	+	
	DNA-based combination vaccines incorporating different stage-specific antigens	+	+			
	Combination vaccines incorporating different stage-specific antigens (e.g., SPf 66)	+	+	+	+	+
Plasmodium vivax	Circumsporozoite antigen-based peptide or recombinant protein	+	+	+		
	Asexual erythrocytic antigens	+	+			
Poliovirus	Reversion-stable attenuated OPV	+				
	Live, attenuated (oral)	+	+	+	+	+
	Inactivated	+	+	+	+	+
	Live (nonreverting)	+	+			
	Chimeric virus	+	+			
	Enhanced potency inactivated	+	+	+	+	+
Pseudomonas aeruginosa	Purified bacterial proteins, including flagellar Ag, LPS-O, porins, several inactivated bacterial toxins, and high MW polysaccharide antigen and glycoconjugate	+	+	+		
	Inactivated whole bacteria—oral preparation	+	+	+		
	Synthetic peptides	+	+	+		
Pseudomonas (Burkholderia) cepacia	Purified bacterial proteins, LPS	+				
Pythium insidiosum	Sonicated hyphal antigens	+	+			
	Culture filtrate antigens	+	+			
	Purified proteins (e.g., 28, 30, 32 kD)	+	+			
Rabies virus	rDNA vaccinia virus recombinant for use in sylvatic rabies (veterinary vaccine)	+	+	+	+	+
	Inactivated mammalian brain	+	+	+	+	+
	Inactivated cell culture	+	+	+	+	+
Respiratory syncytial virus (RSV)	Live, attenuated *ts* and / or *ca* strains	+	+	+		
	Purified F protein subunit vaccine	+	+	+		
	G protein expressed vaccine	+	+			
Rickettsia rickettsii	Subunit vaccine containing major surface proteins (155 and 120 kD)	+	+			
Rift Valley Fever virus	Inactivated	+	+	+	+	
	Live, attenuated	+	+	+		
Rotavirus	Attenuated human / rhesus reassortant viruses	+	+	+	+	+
	Attenuated human rotavirus (cold-adapted)	+	+	+		
	Salmonella expressing VP4, VP7, or both	+	+			
	Attenuated bovine / human virus reassortants (WC3)	+	+	+	+	+
	Human nursery strains	+	+	+	+	
	Purified rotavirus proteins rDNA-derived virus-like particles (VLPs)	+	+			

Target Agent	Vaccine	Basic R&D	Preclinical	Phase I	Phase II	Phase III
Rotavirus (continued)	Vaccinia virus recombinant expressing VP4, VP7, or both	+	+			
	DNA vaccines	+	+			
Rubella virus	Live, attenuated	+	+	+	+	+
	Infectious clone	+				
	Synthetic peptide	+				
Salmonella typhi	Vi carbohydrate	+	+	+	+	+
	Vi carbohydrate-protein conjugate	+	+	+	+	
	Live, attenuated Ty21a vaccine	+	+	+	+	+
	Live, attenuated auxotrophic mutants	+	+	+	+	
Schistosoma mansoni, *Schistosoma haematobium*, *Schistosoma japonicum*	Purified larval antigens	+	+			
	Recombinant larval antigens	+	+			
Shigella dysenteriae	Live auxotrophic, attenuated mutants	+	+	+		
	Polysaccharide-protein conjugate	+	+	+	+	
Shigella flexneri	*E. coli* hybrids	+	+	+	+	
	Polysaccharide-protein conjugate	+	+	+	+	
	Live, attenuated oral vaccines	+	+	+	+	
	LPS proteosome (intranasal)	+	+			
Shigella sonnei	Live, attenuated (WRSS1) oral vaccine	+	+			
	LPS proteosome (intranasal)	+	+			
	Polysaccharide-protein conjugate	+	+	+	+	
	Nucleoprotein	+	+			
Staphylococcus aureus	Type 5/Type capsular polysaccharide (CPS) conjugate with *Pseudomonas aeruginosa* recombinant exoprotein A	+	+	+	+	
Staphylococcal enterotoxin B	Recombinant toxin	+	+			
Streptococcus pneumoniae	Glycoconjugate vaccine (1, 4, 5, 6B, 9N, 14, 18C, 19V, 23F) conjugated to meningococcal B OMP	+	+	+	+	+
	Glycoconjugate vaccine (1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, 23F) conjugated to CRM197	+	+			
	Glycoconjugate vaccine (3, 4, 6B, 9V, 14, 18C, 19F, 23F) conjugated to either tetanus toxoid or diphtheria toxoid	+	+	+	+	
	Glycoconjugate vaccine (6B, 14, 19, 23F) conjugated to tetanus toxoid	+	+	+	+	
	Glycoconjugate vaccine (4, 6B, 9V, 14, 18C, 19F, 23F) conjugated to CRM197	+	+	+	+	+
	Glycoconjugate vaccine (1, 4, 5, 6B, 9V, 14, 18C, 19F, 23F) conjugated to CRM197	+	+	+	+	+
	23-valent licensed vaccine with novel adjuvants (Quil A, QS21, MPL)	+	+	+		
	Glycoconjugate multivalent vaccine with novel adjuvants (e.g., MPL)	+	+	+		
	PspA	+	+	+		
	PsaA	+	+			
	Pneumolysin	+	+			

Target Agent	Vaccine	Basic R&D	Preclinical	Phase I	Phase II	Phase III
Streptococcus pneumoniae (continued)	Autolysin	+	+			
	Neuraminidase	+	+			
	Glycoconjugate vaccine (6B, 14, 19F, 23F) linked to nontypeable *H. influenzae* OMP	+	+	+		
	Glycoconjugate vaccine (1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, 23F) linked to either tetanus or diphtheria toxoid carrier	+	+	+	+	
	Phospholcholine	+	+			
	Synthetic peptide epitopes and capsular polysaccharide combined	+	+			
Tick-borne encephalitis virus	DNA vaccine	+	+			
	Inactivated, alum adjuvant	+	+	+	+	
Toxoplasma gondii	Recombinant parasite surface protein (p30)	+	+			
	Live, attenuated parasites	+	+			
	Parasite surface protein expressed in viral vector	+	+			
Treponema pallidum	Surface lipoproteins	+	+			
	Anti-idiotype/fibronectin	+	+			
Trypanosoma cruzi	Recombinant peptide	+	+			
Varicella zoster virus	Live, attenuated vaccine	+	+	+	+	+
	Subunit, glycoproteins	+				
	Vaccinia-vectored glycoprotein	+				
Venezuelan equine encephalitis	Inactivated, whole virus particles	+	+	+	+	
	Live, attenuated virus strain (TC-83)	+	+	+	+	
	Infectious clones	+	+			
Vibrio cholerae	Killed bacteria plus toxin B subunit	+	+	+	+	+
	Live, recombinant O1	+	+	+	+	+
	Live, recombinant O139	+	+	+	+	
	Conjugate lipopolysaccharide (LPS)	+	+			
Yellow fever virus	Live attenuated	+	+	+	+	+
	Infectious clone	+	+			
Western equine encephalitis virus	Inactivated, whole virus particles	+	+	+	+	
Yersinia pestis	Recombinant subunit	+	+			

APPENDIX B

Licensed Vaccines Currently Distributed in the United States and Recent Revocations, January 1998*

Product Name	Trade Name	License Date	Establishment
Acellular Pertussis Vaccine Concentrate (For Further Manufacturing Use)	No Trade Name	17-Dec-91	Takeda Chemical Industries, Ltd.
Acellular Pertussis Vaccine Concentrate (For Further Manufacturing Use)	No Trade Name	20-Aug-92	Research Fdn. for Microbial Diseases of Osaka University
Adenovirus Vaccine, Live, Oral, Type 4	No Trade Name	01-Jul-80	Wyeth Laboratories, Inc.
Adenovirus Vaccine, Live, Oral, Type 7	No Trade Name	01-Jul-80	Wyeth Laboratories, Inc.
Anthrax Vaccine Adsorbed	No Trade Name	04-Nov-70	Michigan Biologic Products Institute
BCG Live	TheraCys®	21-May-90	Connaught Laboratories Ltd.
BCG Live	Tice® BCG	10-Jan-95	Organon Teknika Corporation
Botulinum Toxin Type A	BOTOX®	09-Dec-91 09-Dec-91	Allergan, Inc.
Cholera Vaccine	No Trade Name	26-Dec-41 23-Oct-96†	Lederle Laboratories Div. of American Cyanamid Co.
Cholera Vaccine	No Trade Name	16-Jul-52	Wyeth Laboratories, Inc.
Diphtheria & Tetanus Toxoids & Acellular Pertussis Vaccine Adsorbed	Tripedia®	20-Aug-92	Connaught Laboratories, Inc.
Diphtheria & Tetanus Toxoids & Acellular Pertussis Vaccine Adsorbed	ACEL-IMUNE®	17-Dec-91	Lederle Laboratories, Div. of American Cyanamid Co.
Diphtheria & Tetanus Toxoids & Pertussis Vaccine Adsorbed	Infanrix®	29-Jan-97 29-Jan-97 22-May-97	SmithKline Beecham Biologicals
Diphtheria & Tetanus Toxoids & Pertussis Vaccine Adsorbed	No Trade Name	11-Sep-70	Wyeth Laboratories, Inc.
Diphtheria & Tetanus Toxoids & Pertussis Vaccine Adsorbed	No Trade Name	03-Jan-78	Connaught Laboratories, Inc.
Diphtheria & Tetanus Toxoids & Pertussis Vaccine Adsorbed	No Trade Name	27-Aug-70	Michigan Biologic Products Institute
Diphtheria & Tetanus Toxoids & Pertussis Vaccine Adsorbed	TRI-IMMUNOL®	24-Jul-70	Lederle Laboratories, Div. of American Cyanamid Co.
Diphtheria & Tetanus Toxoids & Pertussis Vaccine Adsorbed	No Trade Name	27-Jul-70	Massachusetts Public Health Biologic Laboratories
Diphtheria & Tetanus Toxoids & Pertussis Vaccine Adsorbed and Haemophilus b Conjugate Vaccine (Diphtheria CRM197 Protein Conjugate)	TETRAMUNE®	30-Mar-93	Lederle Laboratories, Div. of American Cyanamid Co.
Diphtheria & Tetanus Toxoids Adsorbed	No Trade Name	18-Sep-84	Connaught Laboratories, Inc.
Diphtheria & Tetanus Toxoids Adsorbed	No Trade Name	11-Apr-97	Connaught Laboratories Ltd.
Diphtheria & Tetanus Toxoids Adsorbed	No Trade Name	27-Jul-70	Massachusetts Public Health Biologic Laboratories

* Prepared by Loris McVittie, Ph.D., and Alice D. Knoben, R.Ph., Viral Vaccines Branch, DVRPA, OVRR, CBER. For revisions to this chart, please contact Ms. Knoben at 301-827-3070. Two license dates indicate that the product was licensed to be manufactured at two facilities.

† License revocation date.

Product Name	Trade Name	License Date	Establishment
Diphtheria & Tetanus Toxoids Adsorbed	No Trade Name	27-Aug-70	Michigan Biologic Products Institute
Diphtheria & Tetanus Toxoids Adsorbed	No Trade Name	29-Jul-70	Lederle Laboratories, Div. of American Cyanamid Co.
Diphtheria & Tetanus Toxoids Adsorbed	No Trade Name	11-Sep-70	Wyeth Laboratories, Inc.
Diphtheria Toxoid Adsorbed	No Trade Name	27-Aug-70	Michigan Biologic Products Institute
Haemophilus b Conjugate Vaccine (Diphtheria CRM197 Protein Conjugate)	HibTITER®	06-Dec-94 06-Dec-94	Lederle Laboratories, Div. of American Cyanamid Co.
Haemophilus b Conjugate Vaccine (Diphtheria Toxoid Conjugate)	ProHIBIT®	22-Dec-87	Connaught Laboratories, Inc.
Haemophilus b Conjugate Vaccine (Meningococcal Protein Conjugate)	PedvaxHIB®	20-Dec-89	Merck & Co., Inc.
Haemophilus b Conjugate Vaccine (Tetanus Toxoid Conjugate)	ActHIB® OmniHIB®	30-Mar-93	Pasteur Mérieux Sérums et Vaccins, S.A.
Haemophilus b Conjugate Vaccine (Meningococcal Protein Conjugate) & Hepatitis B (Recombinant) Vaccine	Comvax®	02-Oct-96	Merck & Co., Inc.
Haemophilus b Polysaccharide Vaccine	HibVAX®	20-Dec-85	Connaught Laboratories, Inc.
Haemophilus b Polysaccharide Vaccine	HIB-IMUNE®	20-Dec-85 23-Oct-96†	Lederle Laboratories, Div. of American Cyanamid Co.
Hepatitis A Vaccine, Inactivated	HAVRIX®	22-Feb-95 22-Feb-95	SmithKline Beecham Biologicals
Hepatitis A Vaccine, Inactivated	VAOTA®	29-Mar-96 29-Mar-96	Merck and Co., Inc.
Hepatitis B Vaccine (Recombinant)	HEPTAVAX®	16-Nov-81 15-Mar-95†	Merck & Co., Inc.
Hepatitis B Vaccine (Recombinant)	RECOMBIVAX HB®	23-Jul-86 26-Feb-96	Merck & Co., Inc.
Hepatitis B Vaccine (Recombinant)	Engerix-B®	28-Aug-89 13-Oct-93	SmithKline Beecham Biologicals
Influenza Virus Vaccine	Fluzone®	03-Jan-78	Connaught Laboratories, Inc.
Influenza Virus Vaccine	FluShield®	13-Dec-61	Wyeth Laboratories, Inc.
Influenza Virus Vaccine	Fluogen®	26-Nov-45	Parke-Davis, Div. of Warner-Lambert Co.
Influenza Virus Vaccine	Fluvirin®	12-Aug-88	Evans Medical Ltd.
Influenza Virus Vaccine	FLU-IMUNE®	07-Dec-45	Lederle Laboratories, Div. of American Cyanamid Co.
Japanese Encephalitis Virus Vaccine Inactivated	JE-VAX®	10-Dec-92	Research Fdn. for Microbial Diseases of Osaka University
Measles and Mumps Virus Vaccine Live	M-M-Vax®	18-Jul-73	Merck & Co., Inc.
Measles and Rubella Virus Vaccine Live	M-R-Vax® II	22-Apr-71	Merck & Co., Inc.
Measles, Mumps, and Rubella Virus Vaccine Live	M-M-R® II	22-Apr-71	Merck & Co., Inc.
Measles Virus Vaccine Live	ATTENUVAX®	21-Mar-63	Merck & Co., Inc.
Meningococcal Polysaccharide Vaccine, Group A	No Trade Name	11-Jul-75 15-Mar-95†	Merck & Co., Inc.
Meningococcal Polysaccharide Vaccine, Group A	Menomune®—A	03-Jan-78	Connaught Laboratories, Inc.
Meningococcal Polysaccharide Vaccine, Group C	Menomune®—C	03-Jan-78	Connaught Laboratories, Inc.

Product Name	Trade Name	License Date	Establishment
Meningococcal Polysaccharide Vaccine, Group C	No Trade Name	02-Apr-74 15-Mar-95†	Merck & Co., Inc.
Meningococcal Polysaccharide Vaccine, Groups A and C Combined	No Trade Name	05-Oct-75 15-Mar-95†	Merck & Co., Inc.
Meningococcal Polysaccharide Vaccine, Groups A and C Combined	Menomune®—A/C	03-Jan-78	Connaught Laboratories, Inc.
Meningococcal Polysaccharide Vaccine, Groups A, C, Y and W-135 Combined	Menomune®—A/C/ Y/W-135	23-Nov-81	Connaught Laboratories, Inc.
Meningococcal Polysaccharide Vaccine, Groups A, C, Y and W-135 Combined	No Trade Name	14-Dec-82 15-Mar-95†	Merck & Co., Inc.
Mumps Virus Vaccine Live	MUMPSVAX®	28-Dec-67	Merck & Co., Inc.
Pertussis Vaccine	No Trade Name	03-Jan-78	Connaught Laboratories, Inc.
Pertussis Vaccine Adsorbed	No Trade Name	12-Oct-67	Michigan Biologic Products Institute
Plague Vaccine	No Trade Name	05-Oct-94	Greer Laboratories, Inc.
Pneumococcal Vaccine Polyvalent	PNEUMOVAX® 23	21-Nov-77	Merck & Co., Inc.
Pneumococcal Vaccine Polyvalent	PNU-IMUNE® 23	15-Aug-79 19-Apr-91	Lederle Laboratories, Div. of American Cyanamid Co.
Poliovirus Vaccine Inactivated (Human Diploid Cell)	Poliovax®	20-Nov-87	Connaught Laboratories Ltd.
Poliovirus Vaccine Inactivated (Monkey Kidney Cell)	IPOL®	21-Dec-90	Pasteur Mérieux Sérums et Vaccins, S.A.
Poliovirus Vaccine Live Oral Trivalent (Sabin Strains Types 1, 2 and 3)	ORIMUNE®	25-Jan-63	Lederle Laboratories, Div. of American Cyanamid Co.
Poliovirus Vaccine Live Oral Type I	No Trade Name	27-Mar-62	Lederle Laboratories, Div. of American Cyanamid Co.
Poliovirus Vaccine Live Oral Type II	No Trade Name	27-Mar-62	Lederle Laboratories, Div. of American Cyanamid Co.
Poliovirus Vaccine Live Oral Type III	No Trade Name	27-Mar-62	Lederle Laboratories, Div. of American Cyanamid Co.
Polyvalent Bacterial Antigens with "No U.S. Standard of Potency"	Staphage Lysate®	31-Aug-59	Delmont Laboratories, Inc.
Polyvalent Bacterial Vaccines with "No U.S. Standard of Potency"	MRV® Mixed Respiratory Vaccine	27-Apr-76	Bayer Corporation
Rabies Vaccine	RABIE-VAX®	27-Dec-91	Connaught Laboratories Ltd.
Rabies Vaccine	Imovax® Rabies	09-Jun-80	Pasteur Mérieux Sérums et Vaccins, S.A.
Rabies Vaccine	RabAvert	20-Oct-97	Chiron Behring GmbH & Co.
Rabies Vaccine Adsorbed	No Trade Name	18-Mar-88	Michigan Biologic Products Institute
Rubella and Mumps Virus Vaccine Live	BIAVAX®	30-Aug-70	Merck & Co., Inc.
Rubella Virus Vaccine Live	MERUVAX®	09-Jun-69	Merck & Co., Inc.
Smallpox Vaccine	No Trade Name	03-Jan-78	Connaught Laboratories, Inc.
Smallpox Vaccine	Dryvax®	19-May-44	Wyeth Laboratories, Inc.
Tetanus & Diphtheria Toxoids Adsorbed for Adult Use	No Trade Name	27-Jul-70	Massachusetts Public Health Biologic Laboratories
Tetanus & Diphtheria Toxoids Adsorbed for Adult Use	No Trade Name	03-Jan-78	Connaught Laboratories, Inc.
Tetanus & Diphtheria Toxoids Adsorbed for Adult Use	No Trade Name	11-Sep-70	Wyeth Laboratories, Inc.
Tetanus & Diphtheria Toxoids Adsorbed for Adult Use	No Trade Name	29-Jul-70	Lederle Laboratories, Div. of American Cyanamid Co.

Product Name	Trade Name	License Date	Establishment
Tetanus Toxoid	No Trade Name	03-Jan-78	Connaught Laboratories, Inc.
Tetanus Toxoid	No Trade Name	14-Jan-43	Connaught Laboratories Ltd.
Tetanus Toxoid	No Trade Name	19-May-44	Wyeth Laboratories, Inc.
Tetanus Toxoid Adsorbed	No Trade Name	11-Sep-70	Wyeth Laboratories, Inc.
Tetanus Toxoid Adsorbed	No Trade Name	29-Jul-70	Lederle Laboratories, Div. of American Cyanamid Co.
Tetanus Toxoid Adsorbed	No Trade Name	27-Aug-70	Michigan Biologic Products Institute
Tetanus Toxoid Adsorbed	No Trade Name	11-Dec-70	Swiss Serum and Vaccine Institute Berne
Tetanus Toxoid Adsorbed	No Trade Name	03-Jan-78	Connaught Laboratories, Inc.
Tetanus Toxoid Adsorbed	No Trade Name	29-Jul-70	Massachusetts Public Health Biologic Laboratories
Typhoid Vaccine	No Trade Name	16-Jul-52	Wyeth Laboratories, Inc.
Typhoid Vaccine Live Oral Ty21a	Vivotif Berna®	15-Dec-89	Swiss Serum and Vaccine Institute Berne
Typhoid Vi Polysaccharide Vaccine	Typhim Vi®	28-Nov-94	Pasteur Mérieux Sérums et Vaccins, S.A.
Varicella Virus Vaccine Live	Varivax®	17-Mar-95	Merck & Co., Inc.
Yellow Fever Vaccine	YF-VAX®	03-Jan-78	Connaught Laboratories, Inc.

APPENDIX C

AIDS Vaccine Candidates in Development

Vaccine Candidate	Expression System/ Production Method	HIV Strain(s)	Adjuvant or Delivery System	Stage of Development	References
Subunits:					
rgp160	Baculovirus / insect cell	LAI	alum	PhI-P*, PhI&II-T*	1-11
rgp160	Vaccinia / monkey kidney cell	IIIB	alum + DOC	PhI-P*, PhI&II-T*	12-17
rgp160	Vaccinia / monkey kidney cell	MN	alum + DOC	PhI-P*, PhI&II-T	12, 18
rgp160	Vaccinia / mammalian cell	MN / LAI	alum or IFA	PhI-P	19-24
rgp160	Vaccinia / mammalian cell	LAI	IFA	PhI-P, PhI-T	25-29
rgp160	Vaccinia / mammalian	LAI	Oil / water, 3-deacyl monophosphoryl Lipid A	PCT-C	30
rgp160	Baculovirus / insect cell	MN, Thai Clade E	alum	PCT-SA	1, 31
Oligomeric rgp160	Baculovirus / insect cell	LAI	alum / MPL / IFA	PCT-SA,M	32-36
rgp120(Env 2-3)	Yeast	SF2	MF59 + / - MTP-PE	PhI-P*, PhI-T*	37-40
rgp120	Chinese hamster ovary cells	SF2	MF59 + / - MTP-PE, others	PhI&II-P*, PhI-T*	40-45
rgp120	Chinese hamster ovary cells	IIIB	alum	PhI-P*, PhI-T*	46-50
rgp120	Chinese hamster ovary cells	MN	alum, QS21, QS21 + alum	PhI&II-P*, PhI / II-T*	44, 49-54
rgp120	Chinese hamster ovary cells	MN-Like	QS21 + MPL, alum	PhI-P	29, 55
rgp120	Drosophila	LAI	Oil / water, 3-deacyl monophosphoryl Lipid A	PCT-C	29
rgp120	Mammalian	Thai Clade E (CM235)	MF59	PCT-M	56-57
Bivalent Clade B rgp120	Mammalian	MN / GNE8	alum	PCT-SA	58
Bivalent Clade B / E rgp120	Mammalian	MN / A244	alum	PCT-SA	58
rp24	Baculovirus / insect	LAI	alum	PhI-P*, PhI-T	6-7, 59
rp24	Yeast	SF2	MF59	PhI-P	57, 60
rRT	Recombination	?	cholera toxin	PCT-SA	61
RT-VCG	*E. coli* plasmid / *Vibrio cholerae*	LAI	*V. cholerae* ghosts	PCT-SA	62-63
Tat	Synthetic or recombination			BR&D	64
Peptides:					
V3 peptide	Synthetic peptide	MN	alum or IFA	PhI-P	20-22
V3 peptide (RP400c)	Synthetic	MN	alum	PhI-T	65
Mixed env peptides	Synthetic peptides	LAI	IFA	PhI-P	26
V3-MAPS	Synthetic	MN	alum / microparticulate	PhI-P*	66-70
V3-MAPS	Synthetic	15 strains / 5 clades	alum	PhI-P*	66-67, 71
V3-PPD	V3 peptide coupled to PPD	MN		PhI-P	72-74
V3-PPD	V3 peptide coupled to PPD	5 strains		PhI-P	72
V3-Toxin A	V3 peptide coupled to *Pseudomonas aeruginosa* toxin A	MN	none	PhI-P	72, 75
V3 peptide coupled to Mycobacterium protein	Synthetic	MN	10K Mycobacterium protein	PhI-P	72

* Clinical trial(s) conducted under NIAID sponsorship.

Table adapted and updated August 1997 from: Walker MC, Fast PE. Clinical trials of candidate AIDS vaccines. *AIDS* 1994; 8(Suppl 1):S213-S236.

Vaccine Candidate	Expression System/ Production Method	HIV Strain(s)	Adjuvant or Delivery System	Stage of Development	References
p24-V3 peptide (CLTB-36)	Synthetic chimeric	MN	alum	PhI-P	76-78
V3-T helper epitope peptides (PCLUS 3-18 MN, PCLUS 6-18 MN)	Synthetic chimeric	MN	IFA	PhI-T	79-84
C4-V3 peptides	Synthetic chimeric	MN, RF, CANO, EV91	IFA	PhI-T*, PhI-P*	85-90
Recombinant V3 sequences in single peptide	*E. coli*	Multiple Clade B	?	PhI-P	91-92
Constrained V3 peptide	Synthetic	MN / B consensus		BR&D	93
V3-HA	Recombinant baculovirus	MN	Influenza virus haemagglutinin	PCT-SA	94
HIV-1 gp120-derived multiple chain peptide	Synthetic chimeric	Clade B consensus	CFA / IFA	PCT-SA	95
Multiple cyclic V3 peptides	Synthetic	MN		BR&D	96
Conformationally constrained V3 loop-T helper epitope peptides	Synthetic	Clade B consensus	alum, IFA	PCT-SA	97
V2, V3 peptides	Synthetic	Clade B	ISCOMS	PCT-M	45
Hepatitis B virus surface antigen-gp41 Katinger epitope	Recombinant *Pichia pastoris*	LAI	Hepatitis B virus surface antigen	PCT-SA	98
Multicomponent V3-CD4 binding site-gag peptide (VC1)	Synthetic chimeric	IIIB, Thai A, Thai B, LAI	CFA	PCT-SA	99-101
Multi-*gag, pol, vpu, nef, rev, & tat* CTL epitope peptide (HIV-Peplotion)	Synthetic chimeric	?	Lotion applied to skin or mucous membranes	BR&D	102
CD4 binding domain peptomer	Synthetic, conformationally constrained	MN	Alum	PCT-SA	103
HGP-30	Synthetic p17 peptide	SF2	alum	PhI-P	104-109
gag-lipopeptide	Synthetic	LAI		PhI-P*	110-112
HIV-1 p24-hsp70 fusion protein	Recombination	LAI	*Mycobacterium tuberculosis* heat shock protein (hsp) 70	PCT-SA	113
HIV-1 p24 23mer peptide	Synthetic	Conserved sequence across clades A-G	CFA	PCT-SA	114
Particles:					
p17 / p24:Ty-VLP	Portion p17 / p24 + yeast transposon product	LAI	alum / none	PhI-P*, PhI&II-T	115-121
V3:Ty-VLP	V3-peptide + yeast transposon product	LAI	alum / none	PCT-SA	115, 117, 122-123
Whole inactivated HIV-1, envelope depleted	Inactivated with Betapropiolactone and γ-irradiation	HZ321	IFA	PhIII-T	124-127
Whole inactivated HIV-1, RNA depleted	Stabilized with formaldehyde		Corynebacterium extract Protein 40 and calcium phosphate	PhI-T	128-129
Whole inactivated HIV-1	Inactivated with Betapropiolactone, BEI, formaldehyde	LAI, RF, others	Digitonin	PCT-SA	130-131
HIV-1-*env, gag, protease* Pseudovirions	MoMLV / mammalian cells	LAI, MN, Primary Clade B	CFA, IFA	PCT-SA, M	132-133

Vaccine Candidate	Expression System/ Production Method	HIV Strain(s)	Adjuvant or Delivery System	Stage of Development	References
HIV-1-*env, gag, pol,* Pseudovirions	Vaccinia / mammalian	LAI	alum	PCT-SA, M	134-136
Gag-V3 virus-like particles	Baculovirus / insect cells	LAI		PCT-SA	137-140
P55 Gag particle	Baculovirus / insect cells	LAI	alum	BR&D	141
HBcAg-V3 particles	*E. coli*	LAI		PCT-SA	142
Recombinant live vector:					
Vaccinia-gp160 (HIVAC-1e)	Recombinant vaccinia	LAI		PhI-P*	143-149
Vaccinia-gp160	Recombinant vaccinia	LAI		PhI-P, PhI-T	25-28
Vaccinia-HIV-1 *env, gag, pol* (TBC-3B)	Recombinant vaccinia	LAI		PhI-P*	134-136
Vaccinia-HIV-1 *env, gag, pol* (NYVAC)	Attenuated recombinant vaccinia	LAI, MN		PCT-SA	150-152
Modified vaccinia virus Ankara (MVA)-(SIV) *env, gag, pol*	Attenuated recombinant vaccinia	(SIV)		PCT-M	153-154
Vaccinia-HIV-1 gp140	Recombinant vaccinia	30 isolates		PCT-SA, C, PhI-P	155-156
P55 Gag / V3 chimeric vaccinia	Recombinant vaccinia	LAI	± alum	PCT-SA	157
CP-gp160 (vCP125)	Recombinant canarypox	MN		PhI-P*	20, 23-24, 152, 158-160
CP-*env, gag, protease* (vCP205)	Recombinant canarypox	MN / LAI		PhI&II-P*	77-78, 152, 158-159, 161-163
CP-*env, gag, protease* and other *pol* epitopes, *nef* epitopes (vCP300)	Recombinant canarypox	MN / LAI		PhI-P*	152, 158-159, 164-165
Adenovirus-HIV-1 *env*	Recombinant adenovirus (Ad4, Ad5, Ad7 vaccine strains)	MN		PCT-C	166-168
Poliovirus-HIV-1	Recombinant poliovirus	LAI		BR&D	169-170
Poliovirus-HIV-1 envelope peptides	Recombinant dicistronic poliovirus	LAI		BR&D	171-172
Poliovirus-HIV-1 *nef, gag, env*	Recombinant poliovirus (Mahoney type 1, Sabin types 1 and 2)	multiple		BR&D	173
Encapsidated recombinant poliovirus-HIV-1 *env, gag or pol* minireplicons	Encapsidated recombinant poliovirus	LAI		PCT-SA	174-175
Mengovirus-HIV-1-*nef*	Recombinant mengovirus (attenuated M16 murine strain)	multiple		PCT-SA	176
Mengovirus-HIV-1 V3, C4 peptides	Recombinant murine mengovirus (attenuated)	MN		PCT-SA	177
Rhinovirus-HIV-1 V3, V4 peptides	Recombinant human rhinovirus (HRV14)	multiple		PCT-SA, C	178-179
Influenza-gp41 Katinger epitope	Recombinant influenza virus	LAI		PCT-SA	180-182
Venezuelan equine encephalitis virus-HIV-1 matrix / capsid coding domain	Recombinant Venezuelan equine encephalitis virus	LAI		PCT-SA	183
Simliki Forest virus-(SIV) gp160	Recombinant Simliki Forest virus	(SIV)		PCT-M	184
Simliki Forest virus-HIV-1 envelope (gp120 & gp160)	Recombinant Simliki Forest virus	LAI		BR&D	185

Vaccine Candidate	Expression System/ Production Method	HIV Strain(s)	Adjuvant or Delivery System	Stage of Development	References
VSV-gp160	Recombinant vesicular stomatitis virus	89.6		BR&D	186
V3-*Brucella abortus*	Recombinant *B. abortus*	MN		PCT-SA	187-188
Salmonella-HIV-1 gp120, p24, *nef*	Recombinant *Salmonella typhi* (CVD 908 vaccine strain)	LAI, MN		PCT-SA	189-193
Salmonella-HIV-1 V3 peptide	Recombinant *Salmonella typhimurium* attenuated *aroA* strain	LAI		PCT-SA	194
Salmonella-(SIV) p27	Recombinant *Salmonella typhimurium aroA* mutant	(SIV)		PCT-SA, M	195-196
BCG-HIV-1 *env* peptides	Recombinant BCG	LAI		PCT-SA	197-199
BCG-HIV-1 peptides	Recombinant BCG	LAI		BR&D	200-201
BCG-V3	Recombinant BCG	LAI		PCT-SA	202
BCG-V3	Recombinant BCG	Japanese consensus		PCT-SA	203-204
BCG-(SIV) *nef*	Recombinant BCG	(SIV)		PCT-SA	205
Lactococcus-HIV-1-V3 peptide	Fusion of V3 peptides to TT fragment C in *Lactococcus lactis*	MN		BR&D	206
Lactococcus-HIV-1 peptides	Recombinant *Lactococcus casei* or *Lactococcus lactis*			PCT-SA	207
Listeria-(SIV) gag	Recombinant *Listeria monocytogenes*	(SIV)		PCT-SA	208
Listeria monocytogenes-HIV-1 *gag*	Recombinant *Listeria*	LAI		PCT-SA	209
DNA:					
gp160 + *rev* DNA	Plasmid	MN	bupivacaine	PhI-T*, PhI-P*	210-220
gag + *pol* DNA	Plasmid	LAI	bupivacaine	PhI-P*	210-211, 216-218, 220-221
HIV / DNA expression vector coated 1.0 micron gold particles (gp120, gp160)	Plasmid	LAI	gold particles / gene gun	PCT-SA, M	222-224
gp120 DNA	Plasmid	SF2, CM235 (Thai E), US4 (B)	± gene gun	PCT-SA	225-227
nef, *rev*, *tat*, gp160 DNA	Plasmid	LAI		PCT-SA, PhI-T	228-232
gp120, gp140, gp160 DNA	Plasmid	(SIV)	± gene gun	PCT-SA, M	133-239
gp120 DNA	Plasmid	JR-FL, Bal, HXB2, SP5, SP6	± gene gun	PCT-SA	240
gp120 or *rev*-gp160 DNA	Plasmid	MN, IIIB	± gene gun	PCT-SA, M	241-246
env, *rev* DNA	Plasmid	IIIB		PCT-SA	247-248
gp120 DNA	Plasmid	?	Bupivacaine, Cardiotoxin	PCT-SA	249
gp160 DNA	Plasmid	IIIB	α 25-Dihydroxy-cholecalciferol	PCT-SA	250
Retroviral vectors:					
MoMLV-HIV-1 *env*, *rev* vector	Recombinant murine retrovirus	LAI		PhI-T	251-253
MoMLV-HIV-1 *env*, *rev* vector transduced autologous fibroblasts (retrovector)	Recombinant murine retrovirus / transduction	LAI		PhI-T	251-254
Disrupted autologous HIV+ PBMC & plasma (DROVAC)	Disruption of PBMC	Clade B	Sendi virus envelope derived adjuvant (SDE)	PhI-T	255
CD4 as immunogen:					
Recombinant CD4	Recombinant protein		IFA	PhI-T	256

Vaccine Candidate	Expression System/ Production Method	HIV Strain(s)	Adjuvant or Delivery System	Stage of Development	References
Anti-idiotype approach:					
Anti-gp 120 (C39)	Murine monoclonal antibody		SAF-M	PhI-T	257-259
Anti-CD4 idiotype (IOT4a)	Murine monoclonal antibody		alum	PhI & II-T	260-261
Plant produced:					
Recombinant alfalfa mosaic virus-HIV-1 V3	Recombinant alfalfa mosaic virus / tobacco plants	MN	CFA / IFA	PCT-SA	262
Recombinant cowpea mosaic virus-HIV-1 gp41 (Kennedy epitope)	Recombinant cowpea mosaic virus / plants	LAI		PCT-SA	263
Recombinant cowpea mosaic virus-HIV-1 *env* peptides	Recombinant cowpea mosaic virus / plants			BR&D	264
Live attenuated:					
Live, attenuated HIV	Mutations and deletions	Multiple		PCT-SA, M	265-273
Live, inactivatable, attenuated HIV-1	Insertion of Ganciclovir susceptibility gene into HIV-1, deletion of *nef* gene	Multiple		BR&D	274-275
Live, attenuated SHIV	Recombination, mutation, deletion	Multiple		PCT-M	276-277

Key:

LAI, group of closely related HIV isolates that includes LAV, IIIB, BH10, and BRU
alum, aluminum hydroxide or aluminum phosphate
DOC, deoxycholate
CFA, complete Freund's adjuvant
IFA, incomplete Freund's adjuvant
MF59, microfluidized oil-in-water emulsion
MTP-PE, muramyl tripeptide-phosphatidylethanolamine
PPD, purified protein derivative of *Mycobacterium*
MAPS, multiple antigen presentation system
Ty, yeast retrotransposon
VLP, virus-like particle
MoMLV, Moloney murine leukemia virus
SAF-M, Syntex adjuvant formulation
BCG, Bacillus Calmette-Guérin
BEI, binary ethylenimine
BR&D, Basic Research and Development
PCT, preclinical testing
SA, small animals—mice, guinea pigs, rabbits
M, monkeys—usually macaques
C, chimpanzees
PhI or II or III, phase I or II or III clinical trial
P, prophylactic or preventive
T, therapeutic in HIV-1-infected volunteers

APPENDIX C

References

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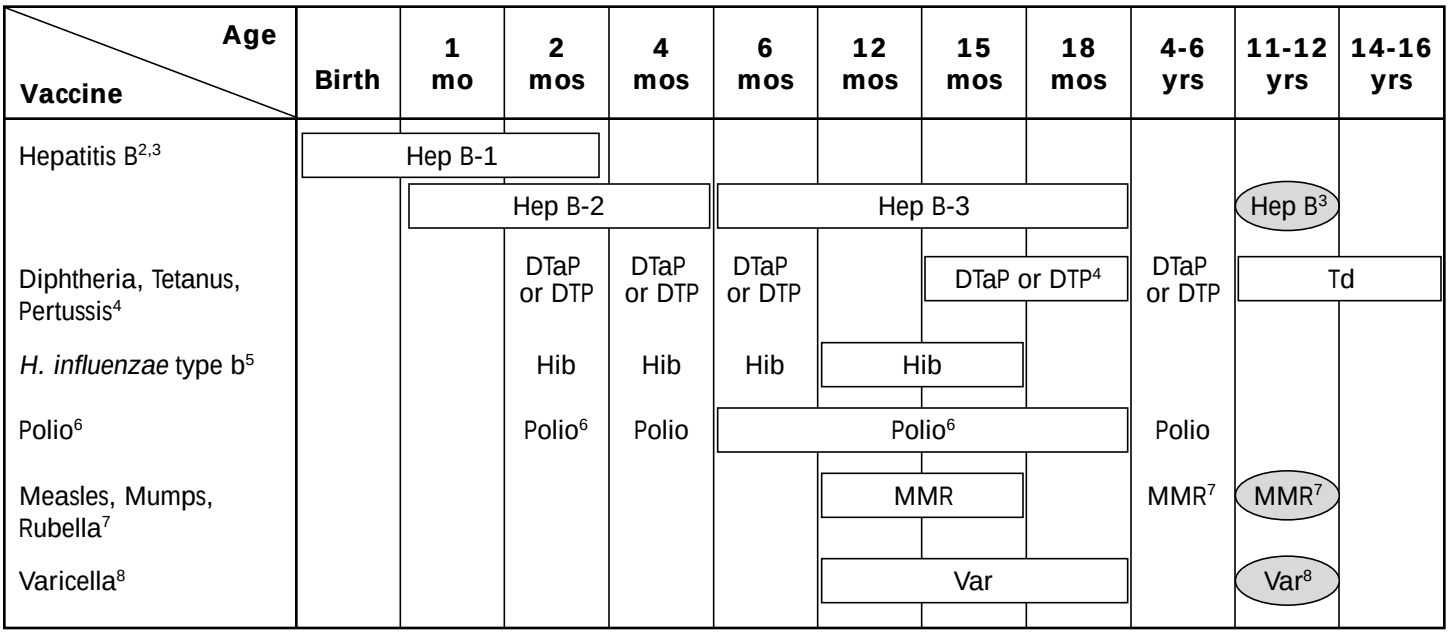
APPENDIX D

Recommended Childhood Immunization Schedule United States, January–December 1998

Vaccines[1] are listed under the routinely recommended ages. Bars indicate range of acceptable ages for immunization. Catch-up immunization should be done during any visit when feasible. Shaded ovals indicate vaccines to be assessed and given if necessary during the early adolescent visit.



Approved by the Advisory Committee on Immunization Practices (ACIP), the American Academy of Pediatrics (AAP), and the American Academy of Family Physicians (AAFP)

[1] This schedule indicates the recommended age for routine administration of currently licensed childhood vaccines. Some combination vaccines are available and may be used whenever administration of all components of the vaccine is indicated. Providers should consult the manufacturers' package inserts for detailed recommendations.

[2] **Infants born to HBsAg-negative mothers** should receive 2.5 µg of Merck vaccine (Recombivax HB®) or 10 µg of SmithKline Beecham (SB) vaccine (Engerix-B®). The second dose should be administered at least 1 month after the first dose. The third dose should be given at least 2 months after the second, but not before 6 months of age.

Infants born to HBsAg-positive mothers should receive 0.5 mL hepatitis B immune globulin (HBIG) with 12 hours of birth and either 5 µg of Merck vaccine (Recombivax HB®) or 10 µg of SB vaccine (Engerix-B®) at a separate site. The second dose is recommended at 1 to 2 months of age and the third dose at 6 months of age.

Infants born to mothers whose HBsAg status is unknown should receive either 5 µg of Merck vaccine (Recombivax HB®) or 10 µg of SB vaccine (Engerix-B®) within 12 hours of birth. The second dose of vaccine is recommended at 1 month of age and the third dose at 6 months of age. Blood should be drawn at the time of delivery to determine the mother's HBsAg status; if it is positive, the infant should receive HBIG as soon as possible (no later than 1 week of age). The dosage and timing of subsequent vaccine doses should be based on the mother's HBsAg status.

[3] Children and adolescents who have not been vaccinated against hepatitis B in infancy may begin the series during any visit. Those who have not previously received three doses of hepatitis B vaccine should initiate or complete the series during the 11- to 12-year-old visit, and unvaccinated older adolescents should be vaccinated whenever possible. The second dose should be administered at least 1 month after the first dose, and the third dose should be administered at least 4 months after the first dose and at least 2 months after the second dose.

[4] DTaP (diphtheria and tetanus toxoids and acellular pertussis vaccine) is the preferred vaccine for all doses in the vaccination series, including completion of the series in children who have received one or more doses of whole-cell DTP vaccine. Whole-cell DTP is an acceptable alternative to DTaP. The fourth dose (DTP or DTaP) may be administered as early as 12 months of age, provided 6 months have elapsed since the third dose and if the child is unlikely to return at age 15 to 18 months. Td (tetanus and diphtheria toxoids) is recommended at 11 to 12 years of age if at least 5 years have elapsed since the last dose of DTP or DTaP, or DT. Subsequent routine Td boosters are recommended every 10 years.

[5] Three *H. influenzae* type b (Hib) conjugate vaccines are licensed for infant use. If PRP-OMP (PedvaxHIB® [Merck]) is administered at 2 and 4 months of age, a dose at 6 months is not required.

[6] Two poliovirus vaccines are currently licensed in the United States: inactivated poliovirus vaccine (IPV) and oral poliovirus vaccine (OPV). The following schedules are all acceptable to the ACIP, the AAP, and the AAFP. Parents and providers may choose among these options.

1. Two doses of IPV followed by two doses of OPV.
2. Four doses of IPV.
3. Four doses of OPV.

The ACIP recommends two doses of IPV at 2 and 4 months of age followed by two doses of OPV at 12 to 18 months and 4 to 6 years of age. IPV is the only poliovirus vaccine recommended for immunocompromised persons and their household contacts.

[7] The second dose of MMR is recommended routinely at 4 to 6 years of age but may be administered during any visit, provided at least 1 month has elapsed since receipt of the first dose and that both doses are administered beginning at or after 12 months of age. Those who have not *previously* received the second dose should complete the schedule no later than the 11- to 12-year-old visit.

[8] Susceptible children may receive Varicella vaccine (Var) at any visit after the first birthday, and those who lack a reliable history of chickenpox should be immunized during the 11- to 12-year-old visit. Susceptible children 13 years of age or older should receive two doses, at least 1 month apart.